

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/605, A61K 38/26	A1	(11) International Publication Number: WO 99/43708 (43) International Publication Date: 2 September 1999 (02.09.99)
(21) International Application Number: PCT/DK99/00086 (22) International Filing Date: 25 February 1999 (25.02.99) (30) Priority Data: 0274/98 27 February 1998 (27.02.98) DK 60/084,357 5 May 1998 (05.05.98) US (71) Applicant (for all designated States except US): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsvaerd (DK). (72) Inventors; and (75) Inventors/Applicants (for US only): KNUDSEN, Liselotte, Bjerre [DK/DK]; Valby Langgade 49A, 1. tv., DK-2500 Valby (DK). HUUSFELDT, Per, Olaf [DK/DK]; Applebys Plads 27,5. mf., DK-1411 Copenhagen K (DK). NIELSEN, Per, Franklin [DK/DK]; Dalsø Park 59, DK-3500 Værløse (DK). MADSEN, Kjeld [DK/DK]; Nyvestergårdsvej 3, DK-3500 Værløse (DK). (74) Common Representative: NOVO NORDISK A/S; Novo Allé, DK-2880 Bagsvaerd (DK).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: GLP-1 DERIVATIVES OF GLP-1 AND EXENDIN WITH PROTRACTED PROFILE OF ACTION		
(57) Abstract <p>The present invention relates to derivatives exendin and of GLP-1(7-C), wherein C is 35 or 36, which derivatives have just one lipophilic substituent which is attached to the C-terminal amino acid residue.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

GLP-1 DERIVATIVES OF GLP-1 AND EXENDIN WITH PROTRACTED PROFILE OF ACTION

FIELD OF THE INVENTION

- 5 The present invention relates to novel derivatives of human glucagon-like peptide-1 (GLP-1) and fragments thereof and analogues of such fragments which have a protracted profile of action and to methods of making and using them. The invention furthermore relates to novel derivatives of exendin and the uses of such derivatives.

10

BACKGROUND OF THE INVENTION

- Peptides are widely used in medical practice, and since they can be produced by recombinant DNA technology it can be expected that their importance will increase also in the years to come. When native peptides or analogues thereof are used in therapy it is generally found that they have a high clearance. A high clearance of a therapeutic agent is inconvenient in cases where it is desired to maintain a high blood level thereof over a prolonged period of time since repeated administrations will then be necessary. Examples of peptides which have a high clearance are: ACTH, corticotropin-releasing factor, angiotensin, calcitonin, insulin, glucagon, glucagon-like peptide-1, glucagon-like peptide-2, insulin-like growth factor-1, insulin-like growth factor-2, gastric inhibitory peptide, growth hormone-releasing factor, pituitary adenylate cyclase activating peptide, secretin, enterogastrin, somatostatin, somatotropin, somatomedin, parathyroid hormone, thrombopoietin, erythropoietin, hypothalamic releasing factors, prolactin, thyroid stimulating hormones, endorphins, enkephalins, vasopressin, oxytocin, opioids and analogues thereof, superoxide dismutase, interferon, asparaginase, arginase, arginine deaminase, adenosine deaminase and ribonuclease. In some cases it is possible to influence the release profile of peptides by applying suitable pharmaceutical compositions, but this approach has various shortcomings and is not generally applicable.

- 30 The hormones regulating insulin secretion belong to the so-called enteroinsular axis, designating a group of hormones, released from the gastrointestinal mucosa in response to the presence and absorption of nutrients in the gut, which promote an early and potentiated release of insulin. The enhancing effect on insulin secretion, the so-called incretin effect, is probably essential for a normal glucose tolerance. Many of the gastrointestinal hormones, including gastrin and secretin (cholecystokinin is not insulinotropic in man), are insulinotro-
- 35

pic, but the only physiologically important ones, those that are responsible for the incretin effect, are the glucose-dependent insulintropic polypeptide, GIP, and glucagon-like peptide-1 (GLP-1). Because of its insulintropic effect, GIP, isolated in 1973 (1) immediately attracted considerable interest among diabetologists. However, numerous investigations carried
5 out during the following years clearly indicated that a defective secretion of GIP was not involved in the pathogenesis of insulin dependent diabetes mellitus (IDDM) or non insulin-dependent diabetes mellitus (NIDDM) (2). Furthermore, as an insulintropic hormone, GIP was found to be almost ineffective in NIDDM (2). The other incretin hormone, GLP-1 is the most potent insulintropic substance known (3). Unlike GIP, it is surprisingly effective in stimulating insulin secretion in NIDDM patients. In addition, and in contrast to the other insulintropic hormones (perhaps with the exception of secretin) it also potently inhibits glucagon secretion. Because of these actions it has pronounced blood glucose lowering effects particularly in patients with NIDDM.

15 GLP-1, a product of the proglucagon (4), is one of the youngest members of the secretin-VIP family of peptides, but is already established as an important gut hormone with regulatory function in glucose metabolism and gastrointestinal secretion and metabolism (5). The glucagon gene is processed differently in the pancreas and in the intestine. In the pancreas (9), the processing leads to the formation and parallel secretion of 1) glucagon itself, occupying
20 positions 33-61 of proglucagon (PG); 2) an N-terminal peptide of 30 amino acids (PG (1-30)) often called glicentin-related pancreatic peptide, GRPP (10, 11); 3) a hexapeptide corresponding to PG (64-69); 4) and, finally, the so-called major proglucagon fragment (PG (72-158)), in which the two glucagon-like sequences are buried (9). Glucagon seems to be the only biologically active product. In contrast, in the intestinal mucosa, it is glucagon that is buried in a larger molecule, while the two glucagon-like peptides are formed separately (8). The
25 following products are formed and secreted in parallel: 1) glicentin, corresponding to PG (1-69), with the glucagon sequence occupying residues Nos. 33-61 (12); 2) GLP-1(7-36)amide (PG (78-107))amide (13), not as originally believed PG (72-107)amide or 108, which is inactive). Small amounts of C-terminally glycine-extended but equally bioactive GLP-1(7-37),
30 (PG (78-108)) are also formed (14); 3) intervening peptide-2 (PG (111-122)amide) (15); and 4) GLP-2 (PG (126-158)) (15, 16). A fraction of glicentin is cleaved further into GRPP (PG (1-30)) and oxyntomodulin (PG (33-69)) (17, 18). Of these peptides, GLP-1, has the most conspicuous biological activities.

35 Being secreted in parallel with glicentin/enteroglucagon, it follows that the many studies of enteroglucagon secretion (6, 7) to some extent also apply to GLP-1 secretion, but GLP-1 is metabolised more quickly with a plasma half-life in humans of 2 min (19). Carbohydrate or fat-rich meals stimulate secretion (20), presumably as a result of direct interaction of yet

unabsorbed nutrients with the microvilli of the open-type L-cells of the gut mucosa. Endocrine or neural mechanisms promoting GLP-1 secretion may exist but have not yet been demonstrated in humans.

- 5 The incretin function of GLP-1(29-31) has been clearly illustrated in experiments with the GLP-1 receptor antagonist, exendin 9-39, which dramatically reduces the incretin effect elicited by oral glucose in rats (21, 22). The hormone interacts directly with the β -cells via the GLP-1 receptor (23) which belongs to the glucagon/VIP/calcitonin family of G-protein-coupled 7-transmembrane spanning receptors. The importance of the GLP-1 receptor in regulating insulin secretion was illustrated in recent experiments in which a targeted disruption of the GLP-1 receptor gene was carried out in mice. Animals homozygous for the disruption had greatly deteriorated glucose tolerance and fasting hyperglycaemia, and even heterozygous animals were glucose intolerant (24). The signal transduction mechanism (25) primarily involves activation of adenylate cyclase, but elevations of intracellular Ca^{2+} are also essential (25, 26). The action of the hormone is best described as a potentiation of glucose stimulated insulin release (25), but the mechanism that couples glucose and GLP-1 stimulation is not known. It may involve a calcium-induced calcium release (26, 27). As already mentioned, the insulinotropic action of GLP-1 is preserved in diabetic β -cells. The relation of the latter to its ability to convey "glucose competence" to isolated insulin-secreting cells (26, 28), which respond poorly to glucose or GLP-1 alone, but fully to a combination of the two, is also not known. Equally importantly, however, the hormone also potently inhibits glucagon secretion (29). The mechanism is not known, but seems to be paracrine, via neighbouring insulin or somatostatin cells (25). Also the glucagonostatic action is glucose-dependent, so that the inhibitory effect decreases as blood glucose decreases. Because of this dual effect, if the plasma GLP-1 concentrations increase either by increased secretion or by exogenous infusion the molar ratio of insulin to glucagon in the blood that reaches the liver via the portal circulation is greatly increased, whereby hepatic glucose production decreases (30). As a result blood glucose concentrations decrease. Because of the glucose dependency of the insulinotropic and glucagonostatic actions, the glucose lowering effect is self-limiting, and the hormone, therefore, does not cause hypoglycaemia regardless of dose (31). The effects are preserved in patients with diabetes mellitus (32), in whom infusions of slightly supraphysiological doses of GLP-1 may completely normalise blood glucose values in spite of poor metabolic control and secondary failure to sulphonylurea (33). The importance of the glucagonostatic effect is illustrated by the finding that GLP-1 also lowers blood glucose in type-1 diabetic patients without residual β -cell secretory capacity (34).

In addition to its effects on the pancreatic islets, GLP-1 has powerful actions on the gastrointestinal tract. Infused in physiological amounts, GLP-1 potently inhibits pentagastrin-

induced as well as meal-induced gastric acid secretion (35, 36). It also inhibits gastric emptying rate and pancreatic enzyme secretion (36). Similar inhibitory effects on gastric and pancreatic secretion and motility may be elicited in humans upon perfusion of the ileum with carbohydrate- or lipid-containing solutions (37, 38). Concomitantly, GLP-1 secretion is greatly stimulated, and it has been speculated that GLP-1 may be at least partly responsible for this so-called "ileal-brake" effect (38). In fact, recent studies suggest that, physiologically, the ileal-brake effects of GLP-1 may be more important than its effects on the pancreatic islets. Thus, in dose response studies GLP-1 influences gastric emptying rate at infusion rates at least as low as those required to influence islet secretion (39).

10

GLP-1 seems to have an effect on food intake. Intraventricular administration of GLP-1 profoundly inhibits food intake in rats (40, 42). This effect seems to be highly specific. Thus, N-terminally extended GLP-1 (PG 72-107)amide is inactive and appropriate doses of the GLP-1 antagonist, exendin 9-39, abolish the effects of GLP-1 (41). Acute, peripheral administration of GLP-1 does not inhibit food intake acutely in rats (41, 42). However, it remains possible that GLP-1 secreted from the intestinal L-cells may also act as a satiety signal.

15

Not only the insulinotropic effects but also the effects of GLP-1 on the gastrointestinal tract are preserved in diabetic patients (43), and may help curtailing meal-induced glucose excursions, but, more importantly, may also influence food intake. Administered intravenously, continuously for one week, GLP-1 at 4 ng/kg/min has been demonstrated to dramatically improve glycaemic control in NIDDM patients without significant side effects (44). The peptide is fully active after subcutaneous administration (45), but is rapidly degraded mainly due to degradation by dipeptidyl peptidase IV-like enzymes (46, 47).

20

The amino acid sequence of GLP-1 is given *i.a.* by Schmidt *et al.* (*Diabetologia* **28** 704-707 (1985)). Although the interesting pharmacological properties of GLP-1(7-37) and analogues thereof have attracted much attention in recent years only little is known about the structure of these molecules. The secondary structure of GLP-1 in micelles has been described by Thorton *et al.* (*Biochemistry* **33** 3532-3539 (1994)), but in normal solution, GLP-1 is considered a very flexible molecule. Surprisingly, we found that derivatisation of this relatively small and very flexible molecule resulted in compounds whose plasma profile were highly protracted and still had retained activity.

25

GLP-1 and analogues of GLP-1 and fragments thereof are potentially useful *i.a.* in the treatment of type 1 and type 2 diabetes. However, the high clearance limits the usefulness of these compounds, and thus there still is a need for improvements in this field. Accordingly, it is one object of the present invention to provide derivatives of GLP-1 and analogues thereof which

30

35

have a protracted profile of action relative to GLP-1(7-37). It is a further object of the invention to provide derivatives of GLP-1 and analogues thereof which have a lower clearance than GLP-1(7-37). It is a further object of the invention to provide a pharmaceutical composition comprising a compound according to the invention and to use a compound of the invention to provide such a composition. Also, it is an object of the present invention to provide a method of treating insulin dependent and non-insulin dependent diabetes mellitus.

References.

1. Pederson RA. Gastric Inhibitory Polypeptide. In Walsh JH, Dockray GJ (eds) Gut peptides: Biochemistry and Physiology. Raven Press, New York 1994, pp. 217-259.
2. Krarup T. Immunoreactive gastric inhibitory polypeptide. *Endocr Rev* 1988;9:122-134.
3. Ørskov C. Glucagon-like peptide-1, a new hormone of the enteroinsular axis. *Diabetologia* 1992; 35:701-711.
4. Bell GI, Sanchez-Pescador R, Laybourn PJ, Najarian RC. Exon duplication and divergence in the human preproglucagon gene. *Nature* 1983; 304: 368-371.
5. Holst JJ. Glucagon-like peptide-1 (GLP-1) - a newly discovered GI hormone. *Gastroenterology* 1994; 107: 1848-1855.
6. Holst JJ. Gut glucagon, enteroglucagon, gut GLI, glicentin - current status. *Gastroenterology* 1983;84:1602-1613.
7. Holst JJ, Ørskov C. Glucagon and other proglucagon-derived peptides. In Walsh JH, Dockray GJ, eds. Gut peptides: Biochemistry and Physiology. Raven Press, New York, pp. 305-340, 1993.
8. Ørskov C, Holst JJ, Knuhtsen S, Baldissera FGA, Poulsen SS, Nielsen OV. Glucagon-like peptides GLP-1 and GLP-2, predicted products of the glucagon gene, are secreted separately from the pig small intestine, but not pancreas. *Endocrinology* 1986;119:1467-1475.

9. Holst JJ, Bersani M, Johnsen AH, Kofod H, Hartmann B, Ørskov C. Proglucagon processing in porcine and human pancreas. *J Biol Chem*, 1994; 269: 18827-1883.
- 5 10. Moody AJ, Holst JJ, Thim L, Jensen SL. Relationship of glicentin to proglucagon and glucagon in the porcine pancreas. *Nature* 1981; 289: 514-516.
11. Thim L, Moody AJ, Purification and chemical characterisation of a glicentin-related pancreatic peptide (proglucagon fragment) from porcine pancreas. *Biochim Biophys Acta*
10 1982;703:134-141.
12. Thim L, Moody AJ. The primary structure of glicentin (proglucagon). *Regul Pept* 1981;2:139-151.
- 15 13. Ørskov C, Bersani M, Johnsen AH, Højrup P, Holst JJ. Complete sequences of glucagon-like peptide-1 (GLP-1) from human and pig small intestine. *J. Biol. Chem.* 1989;264:12826-12829.
14. Ørskov C, Rabenhøj L, Kofod H, Wettergren A, Holst JJ. Production and secretion of
20 amidated and glycine-extended glucagon-like peptide-1 (GLP-1) in man. *Diabetes* 1991; 43: 535-539.
15. Buhl T, Thim L, Kofod H, Ørskov C, Harling H, & Holst JJ: Naturally occurring products of proglucagon 111-160 in the porcine and human small intestine. *J. Biol. Chem.*
25 1988;263:8621-8624.
16. Ørskov C, Buhl T, Rabenhøj L, Kofod H, Holst JJ: Carboxypeptidase-B-like processing of the C-terminus of glucagon-like peptide-2 in pig and human small intestine. *FEBS letters*,
30 1989;247:193-106.
17. Holst JJ. Evidence that enteroglucagon (II) is identical with the C-terminal sequence (residues 33-69) of glicentin. *Biochem J.* 1980;187:337-343.

18. Bataille D, Tatemoto K, Gespach C, Jörnvall H, Rosselin G, Mutt V. Isolation of glucagon-37 (bioactive enteroglucagon/oxyntomodulin) from porcine jejunum-ileum. Characterisation of the peptide. *FEBS Lett* 1982;146:79-86.
- 5 19. Ørskov C, Wettergren A, Holst JJ. The metabolic rate and the biological effects of GLP-1 7-36amide and GLP-1 7-37 in healthy volunteers are identical. *Diabetes* 1993;42:658-661.
- 10 20. Elliott RM, Morgan LM, Tredger JA, Deacon S, Wright J, Marks V. Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. *J Endocrinol* 1993; 138: 159-166.
- 15 21. Kolligs F, Fehmann HC, Göke R, Göke B. Reduction of the incretin effect in rats by the glucagon-like peptide-1 receptor antagonist exendin (9-39)amide. *Diabetes* 1995; 44: 16-19.
- 20 22. Wang Z, Wang RM, Owji AA, Smith DM, Ghatei M, Bloom SR. Glucagon-like peptide-1 is a physiological incretin in rat. *J. Clin. Invest.* 1995; 95: 417-421.
23. Thorens B. Expression cloning of the pancreatic b cell receptor for the gluco-incretin hormone glucagon-like peptide 1. *Proc Natl Acad Sci* 1992;89:8641-4645.
- 25 24. Scrocchi L, Auerbach AB, Joyner AL, Drucker DJ. Diabetes in mice with targeted disruption of the GLP-1 receptor gene. *Diabetes* 1996; 45: 21A.
- 30 25. Fehmann HC, Göke R, Göke B. Cell and molecular biology of the incretin hormones glucagon-like peptide-I (GLP-1) and glucose-dependent insulin releasing polypeptide (GIP). *Endocrine Reviews*, 1995; 16: 390-410.
26. Gromada J, Dissing S, Bokvist K, Renström E, Frøkjær-Jensen J, Wulff BS, Rorsman P. Glucagon-like peptide I increases cytoplasmic calcium in insulin-secreting bTC3-cells by enhancement of intracellular calcium mobilisation. *Diabetes* 1995; 44: 767-774.

27. Holz GG, Leech CA, Habener JF. Activation of a cAMP-regulated Ca^{2+} signaling pathway in pancreatic β -cells by the insulinotropic hormone glucagon-like peptide-1. *J Biol Chem*, 1996; 270: 17749-17759.
- 5 28. Holz GG, Köhlreiter WM, Habener JF. Pancreatic beta-cells are rendered glucose competent by the insulinotropic hormone glucagon-like peptide-1(7-37). *Nature* 1993;361:362-365.
- 10 29. Ørskov C, Holst JJ, Nielsen OV: Effect of truncated glucagon-like peptide-1 (proglucagon 78-107 amide) on endocrine secretion from pig pancreas, antrum and stomach. *Endocrinology* 1988;123:2009-2013.
- 15 30. Hvidberg A, Toft Nielsen M, Hilsted J, Ørskov C, Holst JJ. Effect of glucagon-like peptide-1 (proglucagon 78-107amide) on hepatic glucose production in healthy man. *Metabolism* 1994;43:104-108.
- 20 31. Qualmann C, Nauck M, Holst JJ, Ørskov C, Creutzfeldt W. Insulinotropic actions of intravenous glucagon-like peptide-1 [7-36 amide] in the fasting state in healthy subjects. *Acta Diabetologica*, 1995; 32: 13-16.
32. Nauck MA, Heimesaat MM, Ørskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of GLP-1(7-36amide) but not of synthetic human GIP in patients with type 2-diabetes mellitus. *J Clin Invest* 1993;91:301-307.
- 25 33. Nauck MA, Kleine N, Ørskov C, Holst JJ, Willms B, Creutzfeldt W. Normalisation of fasting hyperglycaemia by exogenous GLP-1(7-36amide) in type 2-diabetic patients. *Diabetologia* 1993;36:741-744.
- 30 34. Creutzfeldt W, Kleine N, Willms B, Ørskov C, Holst JJ, Nauck MA. Glucagonostatic actions and reduction of fasting hyperglycaemia by exogenous glucagon-like peptide-1(7-36amide) in type I diabetic patients. *Diabetes Care* 1996; 19: 580-586.

35. Schjoldager BTG, Mortensen PE, Christiansen J, Ørskov C, Holst JJ. GLP-1 (glucagon-like peptide-1) and truncated GLP-1, fragments of human proglucagon, inhibit gastric acid secretion in man. *Dig. Dis. Sci.* 1989; 35:703-708.
- 5 36. Wettergren A, Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ. Truncated GLP-1 (proglucagon 72-107amide) inhibits gastric and pancreatic functions in man. *Dig Dis Sci* 1993;38:665-673.
37. Layer P, Holst JJ, Grandt D, Goebell H: Ileal release of glucagon-like peptide-1 (GLP-1):
10 association with inhibition of gastric acid in humans. *Dig Dis Sci* 1995; 40: 1074-1082.
38. Layer P, Holst JJ. GLP-1: A humoral mediator of the ileal brake in humans? *Digestion* 1993; 54: 385-386.
- 15 39. Nauck M, Ettler R, Niedereichholz U, Ørskov C, Holst JJ, Schmiegell W. Inhibition of gastric emptying by GLP-1(7-36 amide) or (7-37): effects on postprandial glycaemia and insulin secretion. *Abstract. Gut* 1995; 37 (suppl. 2): A124.
40. Schick RR, vom Walde T, Zimmermann JP, Schusdziarra V, Classen M. Glucagon-like
20 peptide 1 - a novel brain peptide involved in feeding regulation. in Ditschuneit H, Gries FA, Hauner H, Schusdziarra V, Wechsler JG (eds.) *Obesity in Europe*. John Libbey & Company Ltd, 1994; pp. 363-367.
41. Tang-Christensen M, Larsen PJ, Göke R, Fink-Jensen A, Jessop DS, Møller M, Sheikh
25 S. Brain GLP-1(7-36) amide receptors play a major role in regulation of food and water intake. *Am. J. Physiol.*, 1996, in press.
42. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CMB, Meeran K, et al. A role for glucagon-like peptide-1 in the regulation of feeding. *Nature* 1996; 379: 69-72.
- 30 43. Willms B, Werner J, Creutzfeldt W, Ørskov C, Holst JJ, Nauck M. Inhibition of gastric emptying by glucagon-like peptide-1 (7-36 amide) in patients with type-2-diabetes mellitus. *Diabetologia* 1994; 37, suppl.1: A118.

44. Larsen J, Jallad N, Damsbo P. One-week continuous infusion of GLP-1(7-37) improves glycaemic control in NIDDM. *Diabetes* 1996; 45, suppl. 2: 233A.
45. Ritzel R, Ørskov C, Holst JJ, Nauck MA. Pharmacokinetic, insulinotropic, and glucagonostatic properties of GLP-1 [7-36 amide] after subcutaneous injection in healthy volunteers. Dose-response relationships. *Diabetologia* 1995; 38: 720-725.
46. Deacon CF, Johnsen AH, Holst JJ. Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. *J Clin Endocrinol Metab* 1995; 80: 952-957.
47. Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ. 1995. Both subcutaneous and intravenously administered glucagon-like peptide-1 are rapidly degraded from the amino terminus in type II diabetic patients and in healthy subjects. *Diabetes* 44: 1126-1131.

SUMMARY OF THE INVENTION

- Human GLP-1 is a 37 amino acid residue peptide originating from preproglucagon which is synthesised *i.a.* in the L-cells in the distal ileum, in the pancreas and in the brain. Processing of preproglucagon to give GLP-1(7-36)amide, GLP-1(7-37) and GLP-2 occurs mainly in the L-cells. A simple system is used to describe fragments and analogues of this peptide. Thus, for example, Gly⁸-GLP-1(7-37) designates a fragment of GLP-1 formally derived from GLP-1 by deleting the amino acid residues Nos. 1 to 6 and substituting the naturally occurring amino acid residue in position 8 (Ala) by Gly. Similarly, Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-37) designates GLP-1(7-37) wherein the ε-amino group of the Lys residue in position 34 has been tetradecanoylated. Where reference in this text is made to C-terminally extended GLP-1 analogues, the amino acid residue in position 38 is Arg unless otherwise indicated, the optional amino acid residue in position 39 is also Arg unless otherwise indicated and the optional amino acid residue in position 40 is Asp unless otherwise indicated. Also, if a C-terminally extended analogue extends to position 41, 42, 43, 44 or 45, the amino acid sequence of this extension is as in the corresponding sequence in human preproglucagon unless otherwise indicated.

In its broadest aspect, the present invention relates to derivatives of GLP-1 and analogues thereof. The derivatives according to the invention have interesting pharmacological properties, in particular they have a more protracted profile of action than the parent peptides.

- 5 In the present text, the designation "an analogue" is used to designate a peptide wherein one or more amino acid residues of the parent peptide have been substituted by another amino acid residue and/or wherein one or more amino acid residues of the parent peptide have been deleted and/or wherein one or more amino acid residues have been added to the parent peptide. Such addition can take place either at the N-terminal end or at the C-terminal end of the
10 parent peptide or both.

The term "derivative" is used in the present text to designate a peptide in which one or more of the amino acid residues of the parent peptide have been chemically modified, *e.g.* by alkylation, acylation, ester formation or amide formation.

15

The term "a GLP-1 derivative" is used in the present text to designate a derivative of GLP-1 or an analogue thereof. In the present text, the parent peptide from which such a derivative is formally derived is in some places referred to as the "GLP-1 moiety" of the derivative.

- 20 In a preferred embodiment, the present invention relates to a GLP-1 derivative wherein at least one amino acid residue of the parent peptide has a lipophilic substituent attached with the proviso that if only one lipophilic substituent is present and this substituent is attached to the N-terminal or to the C-terminal amino acid residue of the parent peptide then this substituent is an alkyl group or a group which has an ω -carboxylic acid group.

25

In another preferred embodiment, the present invention relates to a GLP-1 derivative having only one lipophilic substituent.

- In another preferred embodiment, the present invention relates to a GLP-1 derivative having
30 only one lipophilic substituent which substituent is an alkyl group or a group which has an ω -carboxylic acid group and is attached to the N-terminal amino acid residue of the parent peptide.

In another preferred embodiment, the present invention relates to a GLP-1 derivative having only one lipophilic substituent which substituent is an alkyl group or a group which has an ω -carboxylic acid group and is attached to the C-terminal amino acid residue of the parent peptide.

5

In another preferred embodiment, the present invention relates to a GLP-1 derivative having only one lipophilic substituent which substituent can be attached to any one amino acid residue which is not the N-terminal or C-terminal amino acid residue of the parent peptide.

10 In another preferred embodiment, the present invention relates to a GLP-1 derivative wherein two lipophilic substituents are present.

In another preferred embodiment, the present invention relates to a GLP-1 derivative wherein two lipophilic substituents are present, one being attached to the N-terminal amino acid residue
15 while the other is attached to the C-terminal amino acid residue.

In another preferred embodiment, the present invention relates to a GLP-1 derivative wherein two lipophilic substituents are present, one being attached to the N-terminal amino acid residue while the other is attached to an amino acid residue which is not N-terminal or the C-terminal
20 amino acid residue.

In another preferred embodiment, the present invention relates to a GLP-1 derivative wherein two lipophilic substituents are present, one being attached to the C-terminal amino acid residue while the other is attached to an amino acid residue which is not the N-terminal or the C-
25 terminal amino acid residue.

In a further preferred embodiment, the present invention relates to a derivative of GLP-1(7-C), wherein C is selected from the group comprising 38, 39, 40, 41, 42, 43, 44 and 45 which derivative has just one lipophilic substituent which is attached to the C-terminal amino acid residue
30 of the parent peptide.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative, being a derivative of GLP-1(7-C), wherein C is 35 or 36 which derivative has just one lipophilic substituent which is attached to the C-terminal amino acid residue.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein the lipophilic substituent comprises from 4 to 40 carbon atoms, more preferred from 8 to 25 carbon atoms.

5

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein a lipophilic substituent is attached to an amino acid residue in such a way that a carboxyl group of the lipophilic substituent forms an amide bond with an amino group of the amino acid residue.

10

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein a lipophilic substituent is attached to an amino acid residue in such a way that an amino group of the lipophilic substituent forms an amide bond with a carboxyl group of the amino acid residue.

15

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein a lipophilic substituent - optionally via a spacer - is attached to the ϵ -amino group of a Lys residue contained in the parent peptide.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an unbranched alkane α,ω -dicarboxylic acid group having from 1 to 7 methylene groups, preferably two methylene groups which spacer forms a bridge between an amino group of the parent peptide and an amino group of the lipophilic substituent.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or a dipeptide such as Gly-Lys. In the present text, the expression "a dipeptide such as Gly-Lys" is used to designate a dipeptide wherein the C-terminal amino acid residue is Lys, His or Trp, preferably Lys, and wherein the N-terminal amino acid residue

30

is selected from the group comprising Ala, Arg, Asp, Asn, Gly, Glu, Gln, Ile, Leu, Val, Phe and Pro.

5 In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys and wherein a carboxyl group of the parent peptide forms an amide bond with an amino group of a Lys residue or a dipeptide containing a Lys residue, and the other amino group of the Lys residue or a dipeptide containing a Lys residue forms an amide bond with a carboxyl group of the lipophilic substituent.

10

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys and wherein an amino group of the parent peptide forms an amide bond with a carboxylic group of the amino acid residue or dipeptide spacer, and an amino group of the amino acid residue or dipeptide spacer forms an amide bond with a carboxyl group of the lipophilic substituent.

15

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys and wherein a carboxyl group of the parent peptide forms an amide bond with an amino group of the amino acid residue spacer or dipeptide spacer, and the carboxyl group of the amino acid residue spacer or dipeptide spacer forms an amide bond with an amino group of the lipophilic substituent.

20

25 In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein a lipophilic substituent is attached to the parent peptide by means of a spacer which is an amino acid residue except Cys, or is a dipeptide such as Gly-Lys, and wherein a carboxyl group of the parent peptide forms an amide bond with an amino group of a spacer which is Asp or Glu, or a dipeptide spacer containing an Asp or Glu residue, and a carboxyl group of the spacer forms an amide bond with an amino group of the lipophilic substituent.

30

In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a lipophilic substituent which comprises a partially or completely hydrogenated cyclopentanophenanthrene skeleton.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a lipophilic substituent which is a straight-chain or branched alkyl group.

- 5 In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a lipophilic substituent which is the acyl group of a straight-chain or branched fatty acid.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a lipophilic substituent which is an acyl group selected from the group comprising $\text{CH}_3(\text{CH}_2)_n\text{CO}-$,
 10 wherein n is an integer from 4 to 38, preferably an integer from 4 to 24, more preferred selected from the group comprising $\text{CH}_3(\text{CH}_2)_6\text{CO}-$, $\text{CH}_3(\text{CH}_2)_8\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{10}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{14}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{16}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{18}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{20}\text{CO}-$ and $\text{CH}_3(\text{CH}_2)_{22}\text{CO}-$.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a
 15 lipophilic substituent which is an acyl group of a straight-chain or branched alkane α,ω -dicarboxylic acid.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a lipophilic substituent which is an acyl group selected from the group comprising
 20 $\text{HOOC}(\text{CH}_2)_m\text{CO}-$, wherein m is an integer from 4 to 38, preferably an integer from 4 to 24, more preferred selected from the group comprising $\text{HOOC}(\text{CH}_2)_{14}\text{CO}-$, $\text{HOOC}(\text{CH}_2)_{16}\text{CO}-$, $\text{HOOC}(\text{CH}_2)_{18}\text{CO}-$, $\text{HOOC}(\text{CH}_2)_{20}\text{CO}-$ and $\text{HOOC}(\text{CH}_2)_{22}\text{CO}-$.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a
 25 lipophilic substituent which is a group of the formula $\text{CH}_3(\text{CH}_2)_p((\text{CH}_2)_q\text{COOH})\text{CHNH}-\text{CO}(\text{CH}_2)_2\text{CO}-$, wherein p and q are integers and p+q is an integer of from 8 to 33, preferably from 12 to 28.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a
 30 lipophilic substituent which is a group of the formula $\text{CH}_3(\text{CH}_2)_r\text{CO-NHCH}(\text{COOH})(\text{CH}_2)_2\text{CO}-$, wherein r is an integer of from 10 to 24.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a lipophilic substituent which is a group of the formula $\text{CH}_3(\text{CH}_2)_s\text{CO-NHCH}((\text{CH}_2)_2\text{COOH})\text{CO-}$, wherein s is an integer of from 8 to 24.

- 5 In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a lipophilic substituent which is a group of the formula $\text{COOH}(\text{CH}_2)_t\text{CO-}$ wherein t is an integer of from 8 to 24.

- In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a
10 lipophilic substituent which is a group of the formula $\text{-NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-CO}(\text{CH}_2)_u\text{CH}_3$, wherein u is an integer of from 8 to 18.

- In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a lipophilic substituent which is a group of the formula $\text{-NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-}$
15 $\text{COCH}((\text{CH}_2)_2\text{COOH})\text{NH-CO}(\text{CH}_2)_w\text{CH}_3$, wherein w is an integer of from 10 to 16.

- In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a lipophilic substituent which is a group of the formula $\text{-NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-}$
20 $\text{CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NH-CO}(\text{CH}_2)_x\text{CH}_3$, wherein x is an integer of from 10 to 16.

- In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a lipophilic substituent which is a group of the formula $\text{-NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-}$
25 $\text{CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NHCO}(\text{CH}_2)_y\text{CH}_3$, wherein y is zero or an integer of from 1 to 22.

- 25 In a further preferred embodiment, the present invention relates to a GLP-1 derivative having a lipophilic substituent which can be negatively charged. Such a lipophilic substituent can for example be a substituent which has a carboxyl group.

- In a further preferred embodiment, the present invention relates to a GLP-1 derivative the parent peptide of which is selected from the group comprising GLP-1(1-45) or an analogue thereof.
30 of.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative derived from a GLP-1 fragment selected from the group comprising GLP-1(7-35), GLP-1(7-36), GLP-

1(7-36)amide, GLP-1(7-37), GLP-1(7-38), GLP-1(7-39), GLP-1(7-40) and GLP-1(7-41) or an analogue thereof.

In a further preferred embodiment, the present invention relates to a GLP-1 analogue derived
5 from a GLP-1 analogue selected from the group comprising GLP-1(1-35), GLP-1(1-36), GLP-1(1-36)amide, GLP-1(1-37), GLP-1(1-38), GLP-1(1-39), GLP-1(1-40) and GLP-1(1-41) or an analogue thereof.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein
10 the designation analogue comprises derivatives wherein a total of up to fifteen, preferably up to ten amino acid residues have been exchanged with any α -amino acid residue.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein the designation analogue comprises derivatives wherein a total of up to fifteen, preferably up to
15 ten amino acid residues have been exchanged with any α -amino acid residue which can be coded for by the genetic code.

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein the designation analogue comprises derivatives wherein a total of up to six amino acid residues
20 have been exchanged with another α -amino acid residue which can be coded for by the genetic code.

In a further preferred embodiment, the present invention relates to a GLP-1(A-B) derivative wherein A is an integer from 1 to 7 and B is an integer from 38 to 45 or an analogue thereof
25 comprising one lipophilic substituent attached to the C-terminal amino acid residue and, optionally, a second lipophilic substituent attached to one of the other amino acid residues.

In a further preferred embodiment, a parent peptide for a derivative according to the invention is selected from the group comprising Arg²⁶-GLP-1(7-37); Arg³⁴-GLP-1(7-37); Lys³⁶-GLP-1(7-
30 37); Arg^{26,34}Lys³⁸-GLP-1(7-37); Arg^{26,34}Lys³⁸GLP-1(7-38); Arg^{26,34}Lys³⁹-GLP-1(7-39); Arg^{26,34}Lys⁴⁰-GLP-1(7-40); Arg²⁶Lys³⁶-GLP-1(7-37); Arg³⁴Lys³⁶-GLP-1(7-37); Arg²⁶Lys³⁹-GLP-1(7-39); Arg³⁴Lys⁴⁰-GLP-1(7-40); Arg^{26,34}Lys^{36,39}-GLP-1(7-39); Arg^{26,34}Lys^{36,40}-GLP-1(7-40); Gly⁸Arg²⁶-GLP-1(7-37); Gly⁸Arg³⁴-GLP-1(7-37); Gly⁸Lys³⁶-GLP-1(7-37); Gly⁸Arg^{26,34}Lys³⁶-GLP-1(7-37); Gly⁸Arg^{26,34}Lys³⁹-GLP-1(7-39); Gly⁸Arg^{26,34}Lys⁴⁰-GLP-1(7-40); Gly⁸Arg²⁶Lys³⁶-GLP-1(7-

37); Gly⁸Arg³⁴Lys³⁶-GLP-1(7-37); Gly⁸Arg²⁶Lys³⁹-GLP-1(7-39); Gly⁸Arg³⁴Lys⁴⁰-GLP-1(7-40); Gly⁸Arg^{26,34}Lys^{36,39}-GLP-1(7-39) and Gly⁸Arg^{26,34}Lys^{36,40}-GLP-1(7-40).

In a further preferred embodiment, a parent peptide for a derivative according to the invention
 5 is selected from the group comprising Arg^{26,34}Lys³⁸GLP-1(7-38); Arg^{26,34}Lys³⁹GLP-1(7-39); Arg^{26,34}Lys⁴⁰GLP-1(7-40); Arg^{26,34}Lys⁴¹GLP-1(7-41); Arg^{26,34}Lys⁴²GLP-1(7-42); Arg^{26,34}Lys⁴³GLP-1(7-43); Arg^{26,34}Lys⁴⁴GLP-1(7-44); Arg^{26,34}Lys⁴⁵GLP-1(7-45); Arg^{26,34}Lys³⁸GLP-1(1-38); Arg^{26,34}Lys³⁹GLP-1(1-39); Arg^{26,34}Lys⁴⁰GLP-1(1-40); Arg^{26,34}Lys⁴¹GLP-1(1-41); Arg^{26,34}Lys⁴²GLP-1(1-42); Arg^{26,34}Lys⁴³GLP-1(1-43); Arg^{26,34}Lys⁴⁴GLP-1(1-44); Arg^{26,34}Lys⁴⁵GLP-1(1-45); Arg^{26,34}Lys³⁸GLP-1(2-38); Arg^{26,34}Lys³⁹GLP-1(2-39); Arg^{26,34}Lys⁴⁰GLP-1(2-40); Arg^{26,34}Lys⁴¹GLP-1(2-41); Arg^{26,34}Lys⁴²GLP-1(2-42); Arg^{26,34}Lys⁴³GLP-1(2-43); Arg^{26,34}Lys⁴⁴GLP-1(2-44); Arg^{26,34}Lys⁴⁵GLP-1(2-45); Arg^{26,34}Lys³⁸GLP-1(3-38); Arg^{26,34}Lys³⁹GLP-1(3-39); Arg^{26,34}Lys⁴⁰GLP-1(3-40); Arg^{26,34}Lys⁴¹GLP-1(3-41); Arg^{26,34}Lys⁴²GLP-1(3-42); Arg^{26,34}Lys⁴³GLP-1(3-43); Arg^{26,34}Lys⁴⁴GLP-1(3-44); Arg^{26,34}Lys⁴⁵GLP-1(3-45); Arg^{26,34}Lys³⁸GLP-1(4-38); Arg^{26,34}Lys³⁹GLP-1(4-39); Arg^{26,34}Lys⁴⁰GLP-1(4-40); Arg^{26,34}Lys⁴¹GLP-1(4-41); Arg^{26,34}Lys⁴²GLP-1(4-42); Arg^{26,34}Lys⁴³GLP-1(4-43); Arg^{26,34}Lys⁴⁴GLP-1(4-44); Arg^{26,34}Lys⁴⁵GLP-1(4-45); Arg^{26,34}Lys³⁸GLP-1(5-38); Arg^{26,34}Lys³⁹GLP-1(5-39); Arg^{26,34}Lys⁴⁰GLP-1(5-40); Arg^{26,34}Lys⁴¹GLP-1(5-41); Arg^{26,34}Lys⁴²GLP-1(5-42); Arg^{26,34}Lys⁴³GLP-1(5-43); Arg^{26,34}Lys⁴⁴GLP-1(5-44); Arg^{26,34}Lys⁴⁵GLP-1(5-45); Arg^{26,34}Lys³⁸GLP-1(6-38); Arg^{26,34}Lys³⁹GLP-1(6-39); Arg^{26,34}Lys⁴⁰GLP-1(6-40); Arg^{26,34}Lys⁴¹GLP-1(6-41); Arg^{26,34}Lys⁴²GLP-1(6-42); Arg^{26,34}Lys⁴³GLP-1(6-43); Arg^{26,34}Lys⁴⁴GLP-1(6-44); Arg^{26,34}Lys⁴⁵GLP-1(6-45); Arg²⁶Lys³⁸GLP-1(1-38); Arg³⁴Lys³⁸GLP-1(1-38); Arg^{26,34}Lys^{36,38}GLP-1(1-38); Arg²⁶Lys³⁸GLP-1(7-38); Arg³⁴Lys³⁸GLP-1(7-38); Arg^{26,34}Lys^{36,38}GLP-1(7-38); Arg^{26,34}Lys³⁸GLP-1(7-38); Arg²⁶Lys³⁹GLP-1(1-39); Arg³⁴Lys³⁹GLP-1(1-39); Arg^{26,34}Lys^{36,39}GLP-1(1-39); Arg²⁶Lys³⁹GLP-1(7-39); Arg³⁴Lys³⁹GLP-1(7-39) and Arg^{26,34}Lys^{36,39}GLP-1(7-39).

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein the parent peptide is selected from the group comprising Arg²⁶-GLP-1(7-37), Arg³⁴-GLP-1(7-37), Lys³⁶-GLP-1(7-37), Arg^{26,34}Lys³⁶-GLP-1(7-37), Arg²⁶Lys³⁸-GLP-1(7-37), Arg³⁴Lys³⁶-GLP-1(7-37), Gly⁸Arg²⁶-GLP-1(7-37), Gly⁸Arg³⁴-GLP-1(7-37), Gly⁸Lys³⁶-GLP-1(7-37), Gly⁸Arg^{26,34}Lys³⁶-GLP-1(7-37), Gly⁸Arg²⁶Lys³⁶-GLP-1(7-37) and Gly⁸Arg³⁴Lys³⁶-GLP-1(7-37).

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein the parent peptide is selected from the group comprising Arg²⁶Lys³⁸-GLP-1(7-38), Arg^{26,34}Lys³⁸-

GLP-1(7-38), Arg^{26,34}Lys^{36,38}-GLP-1(7-38), Gly⁸Arg²⁶Lys³⁸-GLP-1(7-38) and Gly⁸Arg^{26,34}Lys^{36,38}-GLP-1(7-38).

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein
 5 the parent peptide is selected from the group comprising Arg²⁶Lys³⁹-GLP-1(7-39),
 Arg^{26,34}Lys^{36,39}-GLP-1(7-39), Gly⁸Arg²⁶Lys³⁹-GLP-1(7-39) and Gly⁸Arg^{26,34}Lys^{38,39}-GLP-1(7-39).

In a further preferred embodiment, the present invention relates to a GLP-1 derivative wherein
 the parent peptide is selected from the group comprising Arg³⁴Lys⁴⁰-GLP-1(7-40),
 10 Arg^{26,34}Lys^{36,40}-GLP-1(7-40), Gly⁸Arg³⁴Lys⁴⁰-GLP-1(7-40) and Gly⁸Arg^{26,34}Lys^{36,40}-GLP-1(7-40).

In a further preferred embodiment, the present invention relates to a GLP-1 derivative which is
 selected from the group comprising:

- 15 Lys²⁶(N^ε-tetradecanoyl)-GLP-1(7-37);
- Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-37);
- Lys^{26,34}-bis(N^ε-tetradecanoyl)-GLP-1(7-37);
- Gly⁸Lys²⁶(N^ε-tetradecanoyl)-GLP-1(7-37);
- Gly⁸Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-37);
- 20 Gly⁸Lys^{26,34}-bis(N^ε-tetradecanoyl)-GLP-1(7-37);
- Arg²⁶Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-37);
- Lys²⁶(N^ε-tetradecanoyl)-GLP-1(7-38);
- Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-38);
- Lys^{26,34}-bis(N^ε-tetradecanoyl)-GLP-1(7-38);
- 25 Gly⁸Lys²⁶(N^ε-tetradecanoyl)-GLP-1(7-38);
- Gly⁸Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-38);
- Gly⁸Lys^{26,34}-bis(N^ε-tetradecanoyl)-GLP-1(7-38);
- Arg²⁶Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-38);
- Lys²⁶(N^ε-tetradecanoyl)-GLP-1(7-39);
- 30 Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-39);
- Lys^{26,34}-bis(N^ε-tetradecanoyl)-GLP-1(7-39);
- Gly⁸Lys²⁶(N^ε-tetradecanoyl)-GLP-1(7-39);
- Gly⁸Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-39);
- Gly⁸Lys^{26,34}-bis(N^ε-tetradecanoyl)-GLP-1(7-39);

- Arg²⁶Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-39);
Lys²⁶(N^ε-tetradecanoyl)-GLP-1(7-40);
Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-40);
Lys^{26,34}-bis(N^ε-tetradecanoyl)-GLP-1(7-40);
5 Gly⁸Lys²⁶(N^ε-tetradecanoyl)-GLP-1(7-40);
Gly⁸Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-40);
Gly⁸Lys^{26,34}-bis(N^ε-tetradecanoyl)-GLP-1(7-40);
Arg²⁶Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-40);
Lys²⁶(N^ε-tetradecanoyl)-GLP-1(7-36);
10 Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-36);
Lys^{26,34}-bis(N^ε-tetradecanoyl)-GLP-1(7-36);
Gly⁸Lys²⁶(N^ε-tetradecanoyl)-GLP-1(7-36);
Gly⁸Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-36);
Gly⁸Lys^{26,34}-bis(N^ε-tetradecanoyl)-GLP-1(7-36);
15 Arg²⁶Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-36);
Lys²⁶(N^ε-tetradecanoyl)-GLP-1(7-35);
Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-35);
Lys^{26,34}-bis(N^ε-tetradecanoyl)-GLP-1(7-35);
Gly⁸Lys²⁶(N^ε-tetradecanoyl)-GLP-1(7-35);
20 Gly⁸Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-35);
Gly⁸Lys^{26,34}-bis(N^ε-tetradecanoyl)-GLP-1(7-35);
Arg²⁶Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-35);
Lys²⁶(N^ε-tetradecanoyl)-GLP-1(7-36)amide;
Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-36)amide;
25 Lys^{26,34}-bis(N^ε-tetradecanoyl)-GLP-1(7-36)amide;
Gly⁸Lys²⁶(N^ε-tetradecanoyl)-GLP-1(7-36)amide;
Gly⁸Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-36)amide;
Gly⁸Lys^{26,34}-bis(N^ε-tetradecanoyl)-GLP-1(7-36)amide;
Arg²⁶Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-36)amide;
30 Gly⁸Arg²⁶Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-37);
Lys²⁶(N^ε-tetradecanoyl)Arg³⁴-GLP-1(7-37);
Gly⁸Lys²⁶(N^ε-tetradecanoyl)Arg³⁴-GLP-1(7-37);
Arg^{26,34}Lys³⁶(N^ε-tetradecanoyl)-GLP-1(7-37);

- Gly⁸Arg^{26,34}Lys³⁶(N^ε-tetradecanoyl)-GLP-1(7-37);
 Gly⁸Arg²⁶Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-38);
 Lys²⁶(N^ε-tetradecanoyl)Arg³⁴-GLP-1(7-38);
 Gly⁸Lys²⁶(N^ε-tetradecanoyl)Arg³⁴-GLP-1(7-38);
 5 Arg^{26,34}Lys³⁶(N^ε-tetradecanoyl)-GLP-1(7-38);
 Arg^{26,34}Lys³⁶(N^ε-tetradecanoyl)-GLP-1(7-38);
 Gly⁸Arg^{26,34}Lys³⁶(N^ε-tetradecanoyl)-GLP-1(7-38);
 Gly⁸Arg²⁶Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-39);
 Lys²⁶(N^ε-tetradecanoyl)Arg³⁴-GLP-1(7-39);
 10 Gly⁸Lys²⁶(N^ε-tetradecanoyl)Arg³⁴-GLP-1(7-39);
 Arg^{26,34}Lys³⁶(N^ε-tetradecanoyl)-GLP-1(7-39);
 Gly⁸Arg^{26,34}Lys³⁶(N^ε-tetradecanoyl)-GLP-1(7-39);
 Gly⁸Arg²⁶Lys³⁴(N^ε-tetradecanoyl)-GLP-1(7-40);
 Lys²⁶(N^ε-tetradecanoyl)Arg³⁴-GLP-1(7-40);
 15 Gly⁸Lys²⁶(N^ε-tetradecanoyl)Arg³⁴-GLP-1(7-40);
 Arg^{26,34}Lys³⁶(N^ε-tetradecanoyl)-GLP-1(7-40);
 Gly⁸Arg^{26,34}Lys³⁶(N^ε-tetradecanoyl)-GLP-1(7-40);
 Lys²⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-37);
 Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-37);
 20 Lys^{26,34}-bis(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-37);
 Gly⁸Lys²⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-37);
 Gly⁸Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-37);
 Gly⁸Lys^{26,34}-bis(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-37);
 Lys²⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-38);
 25 Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-38);
 Lys^{26,34}-bis(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-38);
 Gly⁸Lys²⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-38);
 Gly⁸Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-38);
 Gly⁸Lys^{26,34}-bis(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-38);
 30 Lys²⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-39);
 Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-39);
 Lys^{26,34}-bis(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-39);
 Gly⁸Lys²⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-39);

- Gly⁸Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-39);
 Gly⁸Lys^{26,34}-bis(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-39);
 Lys²⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-40);
 Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-40);
- 5 Lys^{26,34}-bis(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-40);
 Gly⁸Lys²⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-40);
 Gly⁸Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-40);
 Gly⁸Lys^{26,34}-bis(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-40);
 Lys²⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-36);
- 10 Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-36);
 Lys^{26,34}-bis(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-36);
 Gly⁸Lys²⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-36);
 Gly⁸Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-36);
 Gly⁸Lys^{26,34}-bis(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-36);
- 15 Lys²⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-36)amide;
 Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-36)amide;
 Lys^{26,34}-bis(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-36)amide;
 Gly⁸Lys²⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-36)amide;
 Gly⁸Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-36)amide;
- 20 Gly⁸Lys^{26,34}-bis(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-36)amide;
 Lys²⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-35);
 Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-35);
 Lys^{26,34}-bis(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-35);
 Gly⁸Lys²⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-35);
- 25 Gly⁸Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-35);
 Gly⁸Lys^{26,34}-bis(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-35);
 Arg²⁶Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-37);
 Gly⁸Arg²⁶Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-37);
 Lys²⁶(N^ε-(ω-carboxynonadecanoyl))Arg³⁴-GLP-1(7-37);
- 30 Gly⁸Lys²⁶(N^ε-(ω-carboxynonadecanoyl))Arg³⁴-GLP-1(7-37);
 Arg^{26,34}Lys³⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-37);
 Gly⁸Arg^{26,34}Lys³⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-37);
 Arg²⁶Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-38);

- Gly⁸Arg²⁶Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-38);
 Lys²⁶(N^ε-(ω-carboxynonadecanoyl))Arg³⁴-GLP-1(7-38);
 Gly⁸Lys²⁶(N^ε-(ω-carboxynonadecanoyl))Arg³⁴-GLP-1(7-38);
 Arg^{26,34}Lys³⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-38);
 5 Arg^{26,34}Lys³⁸(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-38);
 Gly⁸Arg^{26,34}Lys³⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-38);
 Arg²⁶Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-39);
 Gly⁸Arg²⁶Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-39);
 Lys²⁶(N^ε-(ω-carboxynonadecanoyl))Arg³⁴-GLP-1(7-39);
 10 Gly⁸Lys²⁶(N^ε-(ω-carboxynonadecanoyl))Arg³⁴-GLP-1(7-39);
 Arg^{26,34}Lys³⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-39);
 Gly⁸Arg^{26,34}Lys³⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-39);
 Arg²⁶Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-40);
 Gly⁸Arg²⁶Lys³⁴(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-40);
 15 Lys²⁶(N^ε-(ω-carboxynonadecanoyl))Arg³⁴-GLP-1(7-40);
 Gly⁸Lys²⁶(N^ε-(ω-carboxynonadecanoyl))Arg³⁴-GLP-1(7-40);
 Arg^{26,34}Lys³⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-40);
 Gly⁸Arg^{26,34}Lys³⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-40);
 Lys²⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-37);
 20 Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-37);
 Lys^{26,34}-bis(N^ε-(7-deoxycholoyl))-GLP-1(7-37);
 Gly⁸Lys²⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-37);
 Gly⁸Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-37);
 Gly⁸Lys^{26,34}-bis(N^ε-(7-deoxycholoyl))-GLP-1(7-37);
 25 Arg²⁶Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-37);
 Lys²⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-38);
 Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-38);
 Lys^{26,34}-bis(N^ε-(7-deoxycholoyl))-GLP-1(7-38);
 Gly⁸Lys²⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-38);
 30 Gly⁸Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-38);
 Gly⁸Lys^{26,34}-bis(N^ε-(7-deoxycholoyl))-GLP-1(7-38);
 Arg²⁶Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-38);
 Lys²⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-39);

- Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-39);
Lys^{26,34}-bis(N^ε-(7-deoxycholoyl))-GLP-1(7-39);
Gly⁸Lys²⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-39);
Gly⁸Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-39);
5 Gly⁸Lys^{26,34}-bis(N^ε-(7-deoxycholoyl))-GLP-1(7-39);
Arg²⁶Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-39);
Lys²⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-40);
Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-40);
Lys^{26,34}-bis(N^ε-(7-deoxycholoyl))-GLP-1(7-40);
10 Gly⁸Lys²⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-40);
Gly⁸Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-40);
Gly⁸Lys^{26,34}-bis(N^ε-(7-deoxycholoyl))-GLP-1(7-40);
Arg²⁶Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-40);
Lys²⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-36);
15 Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-36);
Lys^{26,34}-bis(N^ε-(7-deoxycholoyl))-GLP-1(7-36);
Gly⁸Lys²⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-36);
Gly⁸Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-36);
Gly⁸Lys^{26,34}-bis(N^ε-(7-deoxycholoyl))-GLP-1(7-36);
20 Arg²⁶Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-36);
Lys²⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-35);
Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-35);
Lys^{26,34}-bis(N^ε-(7-deoxycholoyl))-GLP-1(7-35);
Gly⁸Lys²⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-35);
25 Gly⁸Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-35);
Gly⁸Lys^{26,34}-bis(N^ε-(7-deoxycholoyl))-GLP-1(7-35);
Arg²⁶Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-35);
Lys²⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-36)amide;
Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-36)amide;
30 Lys^{26,34}-bis(N^ε-(7-deoxycholoyl))-GLP-1(7-36)amide;
Gly⁸Lys²⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-36)amide;
Gly⁸Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-36)amide;
Gly⁸Lys^{26,34}-bis(N^ε-(7-deoxycholoyl))-GLP-1(7-36)amide;

- Arg²⁶Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-36)amide;
 Gly⁸Arg²⁶Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-37);
 Lys²⁶(N^ε-(7-deoxycholoyl))Arg³⁴-GLP-1(7-37);
 Gly⁸Lys²⁶(N^ε-(7-deoxycholoyl))Arg³⁴-GLP-1(7-37);
 5 Arg^{26,34}Lys³⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-37);
 Gly⁸Arg^{26,34}Lys³⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-37);
 Lys²⁶(N^ε-(choloyl))-GLP-1(7-37);
 Lys³⁴(N^ε-(choloyl))-GLP-1(7-37);
 Lys^{26,34}-bis(N^ε-(choloyl))-GLP-1(7-37);
 10 Gly⁸Lys²⁶(N^ε-(choloyl))-GLP-1(7-37);
 Gly⁸Lys³⁴(N^ε-(choloyl))-GLP-1(7-37);
 Gly⁸Lys^{26,34}-bis(N^ε-(choloyl))-GLP-1(7-37);
 Arg²⁶Lys³⁴(N^ε-(choloyl))-GLP-1(7-37);
 Gly⁸Arg²⁶Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-38);
 15 Lys²⁶(N^ε-(7-deoxycholoyl))Arg³⁴-GLP-1(7-38);
 Gly⁸Lys²⁶(N^ε-(7-deoxycholoyl))Arg³⁴-GLP-1(7-38);
 Arg^{26,34}Lys³⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-38);
 Arg^{26,34}Lys³⁸(N^ε-(7-deoxycholoyl))-GLP-1(7-38);
 Gly⁸Arg^{26,34}Lys³⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-38);
 20 Lys²⁶(N^ε-(choloyl))-GLP-1(7-38);
 Lys³⁴(N^ε-(choloyl))-GLP-1(7-38);
 Lys^{26,34}-bis(N^ε-(choloyl))-GLP-1(7-38);
 Gly⁸Lys²⁶(N^ε-(choloyl))-GLP-1(7-38);
 Gly⁸Lys³⁴(N^ε-(choloyl))-GLP-1(7-38);
 25 Gly⁸Lys^{26,34}-bis(N^ε-(choloyl))-GLP-1(7-38);
 Arg²⁶Lys³⁴(N^ε-(choloyl))-GLP-1(7-38);
 Gly⁸Arg²⁶Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-39);
 Lys²⁶(N^ε-(7-deoxycholoyl))Arg³⁴-GLP-1(7-39);
 Gly⁸Lys²⁶(N^ε-(7-deoxycholoyl))Arg³⁴-GLP-1(7-39);
 30 Arg^{26,34}Lys³⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-39);
 Gly⁸Arg^{26,34}Lys³⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-39);
 Lys²⁶(N^ε-(choloyl))-GLP-1(7-39);
 Lys³⁴(N^ε-(choloyl))-GLP-1(7-39);

- Lys^{26,34}-bis(N^ε-(choloyl))-GLP-1(7-39);
 Gly⁸Lys²⁶(N^ε-(choloyl))-GLP-1(7-39);
 Gly⁸Lys³⁴(N^ε-(choloyl))-GLP-1(7-39);
 Gly⁸Lys^{26,34}-bis(N^ε-(choloyl))-GLP-1(7-39);
 5 Arg²⁶Lys³⁴(N^ε-(choloyl))-GLP-1(7-39);
 Gly⁸Arg²⁶Lys³⁴(N^ε-(7-deoxycholoyl))-GLP-1(7-40);
 Lys²⁶(N^ε-(7-deoxycholoyl))Arg³⁴-GLP-1(7-40);
 Gly⁸Lys²⁶(N^ε-(7-deoxycholoyl))Arg³⁴-GLP-1(7-40);
 Arg^{26,34}Lys³⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-40);
 10 Gly⁸Arg^{26,34}Lys³⁶(N^ε-(7-deoxycholoyl))-GLP-1(7-40);
 Lys²⁶(N^ε-(choloyl))-GLP-1(7-40);
 Lys³⁴(N^ε-(choloyl))-GLP-1(7-40);
 Lys^{26,34}-bis(N^ε-(choloyl))-GLP-1(7-40);
 Gly⁸Lys²⁶(N^ε-(choloyl))-GLP-1(7-40);
 15 Gly⁸Lys³⁴(N^ε-(choloyl))-GLP-1(7-40);
 Gly⁸Lys^{26,34}-bis(N^ε-(choloyl))-GLP-1(7-40);
 Arg²⁶Lys³⁴(N^ε-(choloyl))-GLP-1(7-40);
 Lys²⁶(N^ε-(choloyl))-GLP-1(7-36);
 Lys³⁴(N^ε-(choloyl))-GLP-1(7-36);
 20 Lys^{26,34}-bis(N^ε-(choloyl))-GLP-1(7-36);
 Gly⁸Lys²⁶(N^ε-(choloyl))-GLP-1(7-36);
 Gly⁸Lys³⁴(N^ε-(choloyl))-GLP-1(7-36);
 Gly⁸Lys^{26,34}-bis(N^ε-(choloyl))-GLP-1(7-36);
 Arg²⁶Lys³⁴(N^ε-(choloyl))-GLP-1(7-36);
 25 Lys²⁶(N^ε-(choloyl))-GLP-1(7-35);
 Lys³⁴(N^ε-(choloyl))-GLP-1(7-35);
 Lys^{26,34}-bis(N^ε-(choloyl))-GLP-1(7-35);
 Gly⁸Lys²⁶(N^ε-(choloyl))-GLP-1(7-35);
 Gly⁸Lys³⁴(N^ε-(choloyl))-GLP-1(7-35);
 30 Gly⁸Lys^{26,34}-bis(N^ε-(choloyl))-GLP-1(7-35);
 Arg²⁶Lys³⁴(N^ε-(choloyl))-GLP-1(7-35);
 Lys²⁶(N^ε-(choloyl))-GLP-1(7-36)amide;
 Lys³⁴(N^ε-(choloyl))-GLP-1(7-36)amide;

- Lys^{26,34}-bis(N^ε-(choloyl))-GLP-1(7-36)amide;
Gly⁸Lys²⁶(N^ε-(choloyl))-GLP-1(7-36)amide;
Gly⁸Lys³⁴(N^ε-(choloyl))-GLP-1(7-36)amide;
Gly⁸Lys^{26,34}-bis(N^ε-(choloyl))-GLP-1(7-36)amide;
5 Arg²⁶Lys³⁴(N^ε-(choloyl))-GLP-1(7-36)amide;
Gly⁸Arg²⁶Lys³⁴(N^ε-(choloyl))-GLP-1(7-37);
Lys²⁶(N^ε-(choloyl))Arg³⁴-GLP-1(7-37);
Gly⁸Lys²⁶(N^ε-(choloyl))Arg³⁴-GLP-1(7-37);
Arg^{26,34}Lys³⁶(N^ε-(choloyl))-GLP-1(7-37);
10 Gly⁸Arg^{26,34}Lys³⁶(N^ε-(choloyl))-GLP-1(7-37);
Lys²⁶(N^ε-(lithocholoyl))-GLP-1(7-37);
Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-37);
Lys^{26,34}-bis(N^ε-(lithocholoyl))-GLP-1(7-37);
Gly⁸Lys²⁶(N^ε-(lithocholoyl))-GLP-1(7-37);
15 Gly⁸Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-37);
Gly⁸Lys^{26,34}-bis(N^ε-(lithocholoyl))-GLP-1(7-37);
Arg²⁶Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-37);
Gly⁸Arg²⁶Lys³⁴(N^ε-(choloyl))-GLP-1(7-38);
Lys²⁶(N^ε-(choloyl))Arg³⁴-GLP-1(7-38);
20 Gly⁸Lys²⁶(N^ε-(choloyl))Arg³⁴-GLP-1(7-38);
Arg^{26,34}Lys³⁶(N^ε-(choloyl))-GLP-1(7-38);
Arg^{26,34}Lys³⁸(N^ε-(choloyl))-GLP-1(7-38);
Gly⁸Arg^{26,34}Lys³⁶(N^ε-(choloyl))-GLP-1(7-38);
Lys²⁶(N^ε-(lithocholoyl))-GLP-1(7-38);
25 Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-38);
Lys^{26,34}-bis(N^ε-(lithocholoyl))-GLP-1(7-38);
Gly⁸Lys²⁶(N^ε-(lithocholoyl))-GLP-1(7-38);
Gly⁸Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-38);
Gly⁸Lys^{26,34}-bis(N^ε-(lithocholoyl))-GLP-1(7-38);
30 Arg²⁶Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-38);
Gly⁸Arg²⁶Lys³⁴(N^ε-(choloyl))-GLP-1(7-39);
Lys²⁶(N^ε-(choloyl))Arg³⁴-GLP-1(7-39);
Gly⁸Lys²⁶(N^ε-(choloyl))Arg³⁴-GLP-1(7-39);

- Arg^{26,34}Lys³⁶(N^ε-(choloyl))-GLP-1(7-39);
Gly⁸Arg^{26,34}Lys³⁶(N^ε-(choloyl))-GLP-1(7-39);
Lys²⁶(N^ε-(lithocholoyl))-GLP-1(7-39);
Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-39);
5 Lys^{26,34}-bis(N^ε-(lithocholoyl))-GLP-1(7-39);
Gly⁸Lys²⁶(N^ε-(lithocholoyl))-GLP-1(7-39);
Gly⁸Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-39);
Gly⁸Lys^{26,34}-bis(N^ε-(lithocholoyl))-GLP-1(7-39);
Arg²⁶Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-39);
10 Gly⁸Arg²⁶Lys³⁴(N^ε-(choloyl))-GLP-1(7-40);
Lys²⁶(N^ε-(choloyl))Arg³⁴-GLP-1(7-40);
Gly⁸Lys²⁶(N^ε-(choloyl))Arg³⁴-GLP-1(7-40);
Arg^{26,34}Lys³⁶(N^ε-(choloyl))-GLP-1(7-40);
Gly⁸Arg^{26,34}Lys³⁶(N^ε-(choloyl))-GLP-1(7-40);
15 Lys²⁶(N^ε-(lithocholoyl))-GLP-1(7-40);
Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-40);
Lys^{26,34}-bis(N^ε-(lithocholoyl))-GLP-1(7-40);
Gly⁸Lys²⁶(N^ε-(lithocholoyl))-GLP-1(7-40);
Gly⁸Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-40);
20 Gly⁸Lys^{26,34}-bis(N^ε-(lithocholoyl))-GLP-1(7-40);
Arg²⁶Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-37);
Lys²⁶(N^ε-(lithocholoyl))-GLP-1(7-36);
Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-36);
Lys^{26,34}-bis(N^ε-(lithocholoyl))-GLP-1(7-36);
25 Gly⁸Lys²⁶(N^ε-(lithocholoyl))-GLP-1(7-36);
Gly⁸Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-36);
Gly⁸Lys^{26,34}-bis(N^ε-(lithocholoyl))-GLP-1(7-36);
Arg²⁶Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-36);
Lys²⁶(N^ε-(lithocholoyl))-GLP-1(7-35);
30 Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-35);
Lys^{26,34}-bis(N^ε-(lithocholoyl))-GLP-1(7-35);
Gly⁸Lys²⁶(N^ε-(lithocholoyl))-GLP-1(7-35);
Gly⁸Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-35);

- Gly⁸Lys^{26,34}-bis(N^ε-(lithocholoyl))-GLP-1(7-35);
 Arg²⁶Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-35);
 Lys²⁶(N^ε-(lithocholoyl))-GLP-1(7-36)amide;
 Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-36)amide;
 5 Lys^{26,34}-bis(N^ε-(lithocholoyl))-GLP-1(7-36)amide;
 Gly⁸Lys²⁶(N^ε-(lithocholoyl))-GLP-1(7-36)amide;
 Gly⁸Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-36)amide;
 Gly⁸Lys^{26,34}-bis(N^ε-(lithocholoyl))-GLP-1(7-36)amide;
 Arg²⁶Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-36)amide;
 10 Gly⁸Arg²⁶Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-37);
 Lys²⁶(N^ε-(lithocholoyl))Arg³⁴-GLP-1(7-37);
 Gly⁸Lys²⁶(N^ε-(lithocholoyl))Arg³⁴-GLP-1(7-37);
 Arg^{26,34}Lys³⁶(N^ε-(lithocholoyl))-GLP-1(7-37);
 Arg^{26,34}Lys³⁸(N^ε-(lithocholoyl))-GLP-1(7-37);
 15 Gly⁸Arg^{26,34}Lys³⁶(N^ε-(lithocholoyl))-GLP-1(7-37);
 Gly⁸Arg²⁶Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-38);
 Lys²⁶(N^ε-(lithocholoyl))Arg³⁴-GLP-1(7-38);
 Gly⁸Lys²⁶(N^ε-(lithocholoyl))Arg³⁴-GLP-1(7-38);
 Arg^{26,34}Lys³⁶(N^ε-(lithocholoyl))-GLP-1(7-38);
 20 Arg^{26,34}Lys³⁸(N^ε-(lithocholoyl))-GLP-1(7-38);
 Gly⁸Arg^{26,34}Lys³⁶(N^ε-(lithocholoyl))-GLP-1(7-38);
 Gly⁸Arg²⁶Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-39);
 Lys²⁶(N^ε-(lithocholoyl))Arg³⁴-GLP-1(7-39);
 Gly⁸Lys²⁶(N^ε-(lithocholoyl))Arg³⁴-GLP-1(7-39);
 25 Arg^{26,34}Lys³⁶(N^ε-(lithocholoyl))-GLP-1(7-39);
 Gly⁸Arg^{26,34}Lys³⁶(N^ε-(lithocholoyl))-GLP-1(7-39);
 Gly⁸Arg²⁶Lys³⁴(N^ε-(lithocholoyl))-GLP-1(7-40);
 Lys²⁶(N^ε-(lithocholoyl))Arg³⁴-GLP-1(7-40);
 Gly⁸Lys²⁶(N^ε-(lithocholoyl))Arg³⁴-GLP-1(7-40);
 30 Arg^{26,34}Lys³⁶(N^ε-(lithocholoyl))-GLP-1(7-40) and
 Gly⁸Arg^{26,34}Lys³⁶(N^ε-(lithocholoyl))-GLP-1(7-40).

In a further preferred embodiment, the present invention relates to a pharmaceutical composition comprising a GLP-1 derivative and a pharmaceutically acceptable vehicle or carrier.

In a further preferred embodiment, the present invention relates to the use of a GLP-1 derivative according to the invention for the preparation of a medicament which has a protracted profile of action relative to GLP-1(7-37).

5

In a further preferred embodiment, the present invention relates to the use of a GLP-1 derivative according to the invention for the preparation of a medicament with protracted effect for the treatment of non-insulin dependent diabetes mellitus.

10 In a further preferred embodiment, the present invention relates to the use of a GLP-1 derivative according to the invention for the preparation of a medicament with protracted effect for the treatment of insulin dependent diabetes mellitus.

In a further preferred embodiment, the present invention relates to the use of a GLP-1 derivative according to the invention for the preparation of a medicament with protracted effect for the treatment of obesity.

15 In a further preferred embodiment, the present invention relates to a method of treating insulin dependent or non-insulin dependent diabetes mellitus in a patient in need of such a treatment, comprising administering to the patient a therapeutically effective amount of a GLP-1 derivative of the invention, in particular a derivative of GLP-1(7-C), wherein C is 35 or 36, together with a pharmaceutically acceptable carrier.

20 According to US 5 631 224 (Novo Nordisk A/S) a strong synergistic effect is observed in NIDDM patients by the combined treatment with GLP-1(7-37) or GLP-1(7-36)amide and an oral hypoglycemic agent.

25 Since pharmacodynamic and pharmacokinetic properties can be changed according to patients' demand by selecting a GLP-1 derivative of the present invention, additional therapeutic advantages can be gained by treating the NIDDM patients in a regimen which additionally comprises treatment with another antidiabetic agent.

30

Thus, the invention furthermore relates to the use of a GLP-1 derivative according to the present invention for the preparation of a medicament for use in the treatment of diabetes in a regimen which additionally comprises treatment with another antidiabetic agent.

- 5 In the present context the expression "antidiabetic agent" includes compounds for the treatment and/or prophylaxis of insulin resistance and diseases wherein insulin resistance is the pathophysiological mechanism.

In one embodiment of this invention, the antidiabetic agent is insulin or an analogue or a derivative thereof.

In another embodiment the antidiabetic agent is a hypoglycaemic agent, preferably an oral hypoglycaemic agent.

- 15 Oral hypoglycaemic agents are preferably selected from the group consisting of sulfonylureas, biguanides, thiazolidinediones, glucosidase inhibitors, glucagon antagonists, GLP-1 agonists, potassium channel openers, insulin sensitizers, hepatic enzyme inhibitors, glucose uptake modulators, compounds modifying the lipid metabolism, compounds lowering food intake, and agents acting on the ATP-dependent potassium channel of the β -cells.

20

Among the sulfonylureas, tolbutamide, glibenclamide, glipizide and gliclazide are preferred.

Among the biguanides, metformin is preferred.

Among the thiazolidinediones, troglitazone and ciglitazone are preferred.

Among the glucosidase inhibitors, acarbose is preferred.

- 25 Among the agents acting on the ATP-dependent potassium channel of the β -cells the following are preferred: glibenclamide, glipizide, gliclazide, repaglinide.

US 5424286 describes a method for stimulating insulin release with exendin polypeptide(s).

The exendin polypeptides disclosed include HGEFTFTSDLSKQMEEEEAVRLFIEWLKNNGX;

- 30 wherein X = P or Y, and HX1X2GTFITSDLSKQMEEEEAVRLFIEWLKNNGPSSGAPPPS; wherein X1X2 = SD (exendin-3) or GE (exendin-4)). According to this document the insulinotropic effect of these polypeptides is greater than that attainable by administration of GLP-1.

The exendin-3 and -4 and fragments are useful in treatment of diabetes mellitus (types I or II) and prevention of hyperglycaemia. They normalise hyperglycaemia through glucose-dependent, insulin-independent and insulin-dependent mechanisms. These insulintropic peptides are more active than GLP-1. Exendin-4 is specific for exendin receptors, i.e. it does not
5 interact with vasoactive intestinal peptide receptors.

WO 9746584 describes truncated versions of exendin peptide(s) for treating diabetes. The disclosed peptides increase secretion and biosynthesis of insulin, but reduce those of glucagon. The truncated peptides can be made more economically than full length versions. Compared
10 with GLP-1 and the known exendins, they are more active (effective at lower doses), more stable to degradation and metabolism and have a longer lasting effect.

However, the high clearance limits the usefulness of these compounds, and thus there still is a need for improvements in this field. Accordingly, it is one object of the present invention to provide derivatives of exendin and analogues thereof which have a protracted profile of action relative to native exendin.
15

Thus, in one aspect the invention relates to an exendin derivative wherein at least one amino acid residue of the parent peptide has a lipophilic substituent attached.
20

In a preferred embodiment only one lipophilic substituent is present.

In another preferred embodiment, the lipophilic substituent is attached to the N-terminal amino acid residue.
25

In another preferred embodiment, the lipophilic substituent is attached to the C-terminal amino acid residue.

In another preferred embodiment, the lipophilic substituent is attached to an amino acid residue which is not the N-terminal or C-terminal amino acid residue.
30

In further preferred embodiment, two lipophilic substituents are present.

In another preferred embodiment, one of the lipophilic substituents is attached to the N-terminal amino acid residue while the other is attached to the C-terminal amino acid residue.

5 In another preferred embodiment, one of the lipophilic substituents is attached to the C-terminal amino acid residue while the other is attached to an amino acid residue which is not the N-terminal or C-terminal amino acid residue.

10 In another preferred embodiment, both lipophilic substituents are attached to amino acid residues which are neither the N-terminal nor the C-terminal amino acid residue.

In further preferred embodiment, the lipophilic substituent comprises from 4 to 40 carbon atoms, more preferred from 8 to 25 carbon atoms, such as 12 to 18 carbon atoms.

15 In another preferred embodiment, a lipophilic substituent is attached to an amino acid residue in such a way that a carboxyl group of the lipophilic substituent forms an amide bond with an amino group of the amino acid residue.

20 In another preferred embodiment, a lipophilic substituent is attached to an amino acid residue in such a way that an amino group of the lipophilic substituent forms an amide bond with a carboxyl group of the amino acid residue.

In another preferred embodiment, the lipophilic substituent is attached to the parent peptide by means of a spacer.

25 In another preferred embodiment, the spacer is an unbranched alkane α,ω -dicarboxylic acid group having from 1 to 7 methylene groups, preferably two methylene groups, which form a bridge between an amino group of the parent peptide and an amino group of the lipophilic substituent.

30 In another preferred embodiment, the spacer is an amino acid residue except cys, or a dipeptide such as gly-lys.

In another preferred embodiment, a carboxyl group of the parent peptide forms an amide bond with an amino group of lys or a dipeptide containing a lys residue, and the other amino group of

the lys spacer or a dipeptide spacer containing a lys residue forms an amide bond with a carboxyl group of the lipophilic substituent.

5 In another preferred embodiment, an amino group of the parent peptide forms an amide bond with a carboxylic group of the amino acid residue or dipeptide spacer, and an amino group of the amino acid residue or dipeptide spacer forms an amide bond with a carboxyl group of the lipophilic substituent.

10 In another preferred embodiment, a carboxyl group of the parent peptide forms an amide bond with an amino group of the amino acid residue spacer or dipeptide spacer, and a carboxyl group of the amino acid residue spacer or dipeptide spacer forms an amide bond with an amino group of the lipophilic substituent.

15 In another preferred embodiment, a carboxyl group of the parent peptide forms an amide bond with an amino group of a spacer which is asp or glu, or a dipeptide spacer containing an asp or glu residue, and a carboxyl group of the spacer forms an amide bond with an amino group of the lipophilic substituent.

20 In one embodiment said spacer is γ -aminobutyroyl.

In a further preferred embodiment, the lipophilic substituent comprises a partially or completely hydrogenated cyclopentanophenathrene skeleton.

25 In another preferred embodiment, the lipophilic substituent is an straight-chain or branched alkyl group.

In another preferred embodiment, the lipophilic substituent is the acyl group of a straight-chain or branched fatty acid.

30 In another preferred embodiment, the acyl group is selected from the group comprising $\text{CH}_3(\text{CH}_2)_n\text{CO}-$, wherein n is 4 to 38, preferably $\text{CH}_3(\text{CH}_2)_6\text{CO}-$, $\text{CH}_3(\text{CH}_2)_8\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{10}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{14}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{16}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{18}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{20}\text{CO}-$ and $\text{CH}_3(\text{CH}_2)_{22}\text{CO}-$, most preferably hexadecanoyl.

In another preferred embodiment, the lipophilic substituent is an acyl group of a straight-chain or branched alkane α,ω -dicarboxylic acid.

In another preferred embodiment, the acyl group is selected from the group comprising
 5 $\text{HOOC}(\text{CH}_2)_m\text{CO}-$, wherein m is from 4 to 38, preferably from 4 to 24, more preferred selected from the group comprising $\text{HOOC}(\text{CH}_2)_{14}\text{CO}-$, $\text{HOOC}(\text{CH}_2)_{16}\text{CO}-$, $\text{HOOC}(\text{CH}_2)_{18}\text{CO}-$, $\text{HOOC}(\text{CH}_2)_{20}\text{CO}-$ and $\text{HOOC}(\text{CH}_2)_{22}\text{CO}-$.

In another preferred embodiment, the lipophilic substituent is a group of the formula
 10 $\text{CH}_3(\text{CH}_2)_p((\text{CH}_2)_q\text{COOH})\text{CHNH-CO}(\text{CH}_2)_2\text{CO}-$, wherein p and q are integers and $p+q$ is an integer of from 8 to 33, preferably from 12 to 28.

In another preferred embodiment, the lipophilic substituent is a group of the formula
 15 $\text{CH}_3(\text{CH}_2)_r\text{CO-NHCH}(\text{COOH})(\text{CH}_2)_2\text{CO}-$, wherein r is an integer of from 10 to 24.

In another preferred embodiment, the lipophilic substituent is a group of the formula
 $\text{CH}_3(\text{CH}_2)_s\text{CO-NHCH}((\text{CH}_2)_2\text{COOH})\text{CO}-$, wherein s is an integer of from 8 to 24.

In another preferred embodiment, the lipophilic substituent is a group of the formula
 20 $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-CO}(\text{CH}_2)_u\text{CH}_3$, wherein u is an integer of from 8 to 18.

In another preferred embodiment, the lipophilic substituent is a group of the formula
 $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-COCH}((\text{CH}_2)_2\text{COOH})\text{NH-CO}(\text{CH}_2)_w\text{CH}_3$, wherein w is an integer of from 10 to 16.

25 In another preferred embodiment, the lipophilic substituent is a group of the formula
 $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NH-CO}(\text{CH}_2)_x\text{CH}_3$, wherein x is an integer of from 10 to 16.

30 In another preferred embodiment, the lipophilic substituent is a group of the formula
 $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NH-CO}(\text{CH}_2)_y\text{CH}_3$, wherein y is zero or an integer of from 1 to 22.

In another preferred embodiment, the designation analogue comprises derivatives wherein a total of up to fifteen, preferably up to ten amino acid residues have been exchanged with any α -amino acid residue.

- 5 In another preferred embodiment, the designation analogue comprises derivatives wherein a total of up to fifteen, preferably up to ten amino acid residues have been exchanged with any α -amino acid residue which can be coded for by the genetic code.

- 10 In another preferred embodiment, the designation analogue comprises derivatives wherein a total of up to six amino acid residues have been exchanged with any α -amino acid residue which can be coded for by the genetic code.

- In another preferred embodiment, the parent peptide is
HGEGTFTSDLSKQMEEEAVRLFIEWLKNNGGX, wherein X = P or Y, or a fragment or an ana-
15 logue thereof.

- In another preferred embodiment, the parent peptide is
HX1X2GTFITSDLSKQMEEEAVRLFIEWLKNNGPSSGAPPPS, wherein X1X2 = SD or GE, or
a fragment or an analogue thereof.
20

In another preferred embodiment, the parent peptide is
DLSKQMEEEAVRLFIEWLKNNGPSSGAPPPS, or a fragment or an analogue thereof.

- In another preferred embodiment the exendin derivative is selected from
25 Arg¹⁸, Leu²⁰, Gln³⁴, Lys³³ (N^ε-(γ -aminobutyroyl(N⁶-hexadecanoyl))) Exendin-4-(7-45)-NH₂,
Arg³³, Leu²⁰, Gln³⁴, Lys¹⁸ (N^ε-(γ -aminobutyroyl(N⁶-hexadecanoyl))) Exendin-4-(7-45)-NH₂.

- The present invention furthermore relates to a pharmaceutical composition comprising an
exendin derivative according to the present invention and a pharmaceutically acceptable ve-
30 hicle or carrier.

Moreover, the invention is concerned with the use of an exendin derivative according to the
present invention for the preparation of a medicament which has a protracted profile of action
relative to exendin.

The invention also relates to the use of an exendin derivative according to the present invention for the preparation of a medicament with a protracted profile of action for the treatment of non-insulin dependent diabetes mellitus or for the treatment of insulin dependent diabetes mellitus or for the treatment of obesity.

The invention also relates to a method of treating insulin dependent or non-insulin dependent diabetes mellitus in a patient in need of such a treatment, comprising administering to the patient a therapeutically effective amount of an exendin derivative according to the present invention together with a pharmaceutically acceptable carrier.

DETAILED DESCRIPTION OF THE INVENTION

To obtain a satisfactory protracted profile of action of the GLP-1 derivative, the lipophilic substituent attached to the GLP-1 moiety preferably comprises 4-40 carbon atoms, in particular 8-25 carbon atoms. The lipophilic substituent may be attached to an amino group of the GLP-1 moiety by means of a carboxyl group of the lipophilic substituent which forms an amide bond with an amino group of the amino acid residue to which it is attached. Alternatively, the lipophilic substituent may be attached to said amino acid residue in such a way that an amino group of the lipophilic substituent forms an amide bond with a carboxyl group of the amino acid residue. As a further option, the lipophilic substituent may be linked to the GLP-1 moiety via an ester bond. Formally, the ester can be formed either by reaction between a carboxyl group of the GLP-1 moiety and a hydroxyl group of the substituent-to-be or by reaction between a hydroxyl group of the GLP-1 moiety and a carboxyl group of the substituent-to-be. As a further alternative, the lipophilic substituent can be an alkyl group which is introduced into a primary amino group of the GLP-1 moiety.

In one preferred embodiment of the invention, the lipophilic substituent is attached to the GLP-1 moiety by means of a spacer in such a way that a carboxyl group of the spacer forms an amide bond with an amino group of the GLP-1 moiety. Examples of suitable spacers are succinic acid, Lys, Glu or Asp, or a dipeptide such as Gly-Lys. When the spacer is succinic acid, one carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the other carboxyl group thereof may form an amide bond with an amino group of

the lipophilic substituent. When the spacer is Lys, Glu or Asp, the carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the amino group thereof may form an amide bond with a carboxyl group of the lipophilic substituent. When Lys is used as the spacer, a further spacer may in some instances be inserted between the ϵ -amino group of Lys and the lipophilic substituent. In one preferred embodiment, such a further spacer is succinic acid which forms an amide bond with the ϵ -amino group of Lys and with an amino group present in the lipophilic substituent. In another preferred embodiment such a further spacer is Glu or Asp which forms an amide bond with the ϵ -amino group of Lys and another amide bond with a carboxyl group present in the lipophilic substituent, that is, the lipophilic substituent is a N⁶-acylated lysine residue.

In another preferred embodiment of the present invention, the lipophilic substituent has a group which can be negatively charged. One preferred group which can be negatively charged is a carboxylic acid group.

15

The parent peptide can be produced by a method which comprises culturing a host cell containing a DNA sequence encoding the polypeptide and capable of expressing the polypeptide in a suitable nutrient medium under conditions permitting the expression of the peptide, after which the resulting peptide is recovered from the culture.

20

The medium used to culture the cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection). The peptide produced by the cells may then be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. ion exchange chromatography, gel filtration chromatography, affinity chromatography, or the like, dependent on the type of peptide in question.

30

The DNA sequence encoding the parent peptide may suitably be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the peptide by hybridisation using synthetic oligonucleotide probes

in accordance with standard techniques (see, for example, Sambrook, J, Fritsch, EF and Maniatis, T, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, 1989). The DNA sequence encoding the peptide may also be prepared synthetically by established standard methods, e.g. the phosphoramidite method described by Beaucage and Caruthers, *Tetrahedron Letters* **22** (1981), 1859 - 1869, or the method described by Matthes *et al.*, *EMBO Journal* **3** (1984), 801 - 805. The DNA sequence may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202 or Saiki *et al.*, *Science* **239** (1988), 487 - 491.

- 10 The DNA sequence may be inserted into any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, *i.e.* a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

The vector is preferably an expression vector in which the DNA sequence encoding the peptide is operably linked to additional segments required for transcription of the DNA, such as a promoter. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA encoding the peptide of the invention in a variety of host cells are well known in the art, cf. for instance Sambrook *et al.*, *supra*.

25 The DNA sequence encoding the peptide may also, if necessary, be operably connected to a suitable terminator, polyadenylation signals, transcriptional enhancer sequences, and translational enhancer sequences. The recombinant vector of the invention may further comprise a DNA sequence enabling the vector to replicate in the host cell in question.

30 The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell or one which confers resistance to a drug, e.g. ampicillin, kanamycin, tetracyclin, chloramphenicol, neomycin, hygromycin or methotrexate.

To direct a parent peptide of the present invention into the secretory pathway of the host cells, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) may be provided in the recombinant vector. The secretory signal sequence is joined to the DNA sequence encoding the peptide in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the peptide. The secretory signal sequence may be that normally associated with the peptide or may be from a gene encoding another secreted protein.

The procedures used to ligate the DNA sequences coding for the present peptide, the promoter and optionally the terminator and/or secretory signal sequence, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (cf., for instance, Sambrook *et al.*, *supra*).

The host cell into which the DNA sequence or the recombinant vector is introduced may be any cell which is capable of producing the present peptide and includes bacteria, yeast, fungi and higher eukaryotic cells. Examples of suitable host cells well known and used in the art are, without limitation, *E. coli*, *Saccharomyces cerevisiae*, or mammalian BHK or CHO cell lines.

Examples of compounds which can be useful as GLP-1 moieties according to the present invention are described in International Patent Application No. WO 87/06941 (The General Hospital Corporation) which relates to a peptide fragment which comprises GLP-1(7-37) and functional derivatives thereof and to its use as an insulinotropic agent.

Further GLP-1 analogues are described in International Patent Application No. 90/11296 (The General Hospital Corporation) which relates to peptide fragments which comprise GLP-1(7-36) and functional derivatives thereof and have an insulinotropic activity which exceeds the insulinotropic activity of GLP-1(1-36) or GLP-1(1-37) and to their use as insulinotropic agents.

International Patent Application No. 91/11457 (Buckley *et al.*) discloses analogues of the active GLP-1 peptides 7-34, 7-35, 7-36, and 7-37 which can also be useful as GLP-1 moieties according to the present invention.

Pharmaceutical compositions

Pharmaceutical compositions containing a GLP-1 derivative according to the present invention may be administered parenterally to patients in need of such a treatment. Parenteral administration may be performed by subcutaneous, intramuscular or intravenous injection by means of a syringe, optionally a pen-like syringe. Alternatively, parenteral administration can be performed by means of an infusion pump. A further option is a composition which may be a powder or a liquid for the administration of the GLP-1 derivative in the form of a nasal or pulmo-
5 nal spray. As a still further option, the GLP-1 derivatives of the invention can also be administered transdermally, e.g. from a patch, optionally a iontophoretic patch, or transmucosally, e.g. bu-
10 cally.

Pharmaceutical compositions containing a GLP-1 derivative of the present invention may be prepared by conventional techniques, e.g. as described in Remington's *Pharmaceutical Sciences*, 1985 or in Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

15

Thus, the injectable compositions of the GLP-1 derivative of the invention can be prepared using the conventional techniques of the pharmaceutical industry which involves dissolving and mixing the ingredients as appropriate to give the desired end product.

20 According to one procedure, the GLP-1 derivative is dissolved in an amount of water which is somewhat less than the final volume of the composition to be prepared. An isotonic agent, a preservative and a buffer is added as required and the pH value of the solution is adjusted - if necessary - using an acid, e.g. hydrochloric acid, or a base, e.g. aqueous sodium hydroxide as needed. Finally, the volume of the solution is adjusted with water to give the desired concen-
25 tration of the ingredients.

Examples of isotonic agents are sodium chloride, mannitol and glycerol.

Examples of preservatives are phenol, m-cresol, methyl p-hydroxybenzoate and benzyl alco-
30 hol.

Examples of suitable buffers are sodium acetate and sodium phosphate.

Further to the above-mentioned components, solutions containing a GLP-1 derivative according to the present invention may also contain a surfactant in order to improve the solubility and/or the stability of the GLP-1 derivative.

- 5 A composition for nasal administration of certain peptides may, for example, be prepared as described in European Patent No. 272097 (to Novo Nordisk A/S) or in WO 93/18785.

According to one preferred embodiment of the present invention, the GLP-1 derivative is provided in the form of a composition suitable for administration by injection. Such a composition
10 can either be an injectable solution ready for use or it can be an amount of a solid composition, e.g. a lyophilised product, which has to be dissolved in a solvent before it can be injected. The injectable solution preferably contains not less than about 2 mg/ml, preferably not less than about 5 mg/ml, more preferred not less than about 10 mg/ml of the GLP-1 derivative and, preferably, not more than about 100 mg/ml of the GLP-1 derivative.

15

The GLP-1 derivatives of this invention can be used in the treatment of various diseases. The particular GLP-1 derivative to be used and the optimal dose level for any patient will depend on the disease to be treated and on a variety of factors including the efficacy of the specific peptide derivative employed, the age, body weight, physical activity, and diet of the patient, on a
20 possible combination with other drugs, and on the severity of the case. It is recommended that the dosage of the GLP-1 derivative of this invention be determined for each individual patient by those skilled in the art.

In particular, it is envisaged that the GLP-1 derivative will be useful for the preparation of a medicament with a protracted profile of action for the treatment of non-insulin dependent diabetes
25 mellitus and/or for the treatment of obesity.

The present invention is further illustrated by the following examples which, however, are not to be construed as limiting the scope of protection. The features disclosed in the foregoing description and in the following examples may, both separately and in any combination thereof,
30 be material for realising the invention in diverse forms thereof.

EXAMPLES

The following acronyms for commercially available chemicals are used:

	DMF	:	N,N-Dimethylformamide.
	DCC	:	N,N-Dicyclohexylcarbodiimide
	NMP	:	N-Methyl-2-pyrrolidone.
	EDPA	:	N-Ethyl-N,N-diisopropylamine.
5	EGTA	:	Ethylene glycol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid.
	GTP		Guanosine 5'-triphosphate.
	TFA	:	Trifluoroacetic acid.
	THF	:	Tetrahydrofuran
	H-Glu(OH)-OBu ^t :		L-Glutamic acid α -tert-butyl ester
10	Cap-ONSu:		Octanoic acid 2,5-dioxopyrrolidin-1-yl ester
	Lau-ONSu:		Dodecanoic acid 2,5-dioxopyrrolidin-1-yl ester
	Myr-ONSu:		Tetradecanoic acid 2,5-dioxopyrrolidin-1-yl ester.
	Pal-ONSu:		Hexadecanoic acid 2,5-dioxopyrrolidin-1-yl ester.
	Ste-ONSu		Octadecanoic acid 2,5-dioxopyrrolidin-1-yl ester.

15

Abbreviations:

PDMS: Plasma Desorption Mass Spectrometry

MALDI-MS: Matrix Assisted Laser Desorption/Ionisation Mass Spectrometry

HPLC: High Performance Liquid Chromatography

20 amu: atomic mass units

Lit-Glu(ONSu)-OBu^t: N ^{α} -Lithochoyl-L-glutamic acid α -t-butyl ester γ -2,5-dioxopyrrolidin-1-yl esterCap-Glu(ONSu)-OBu^t: N ^{α} -Octanoyl-L-glutamic acid α -t-butyl ester γ -2,5-dioxopyrrolidin-1-yl ester25 Cac-Glu(ONSu)-OBu^t: N ^{α} -Decanoyl-L-glutamic acid α -t-butyl ester γ -2,5-dioxopyrrolidin-1-yl esterLau-Glu(ONSu)-OBu^t: N ^{α} -Dodecanoyl-L-glutamic acid α -t-butyl ester γ -2,5-dioxopyrrolidin-1-yl ester30 Myr-Glu(ONSu)-OBu^t: N ^{α} -Tetradecanoyl-L-glutamic acid α -t-butyl ester γ -2,5-dioxopyrrolidin-1-yl esterPal-Glu(ONSu)-OBu^t: N ^{α} -Hexadecanoyl-(L)-glutamic acid α -t-butyl- γ -2,5-dioxopyrrolidin-1-yl diester.Ste-Glu(ONSu)-OBu^t: N ^{α} -Octadecanoyl-(L)-glutamic acid α -t-butyl- γ -2,5-dioxopyrrolidin-1-yl diester

- Lau- β -Ala-ONSu: N ^{β} -Dodecanoyl- β -alanine 2,5-dioxopyrrolidin-1-yl ester
 Pal- β -Ala-ONSu: N ^{β} -Hexadecanoyl- β -alanine 2,5-dioxopyrrolidin-1-yl ester
 Lau-GABA-ONSu: N ^{γ} -Dodecanoyl- γ -aminobutyric acid 2,5-dioxopyrrolidin-1-yl ester
 Myr-GABA-ONSu: N ^{γ} -Tetradecanoyl- γ -aminobutyric acid 2,5-dioxopyrrolidin-1-yl ester
 5 Pal-GABA-ONSu: N ^{γ} -Hexadecanoyl- γ -aminobutyric acid 2,5-dioxopyrrolidin-1-yl ester
 Ste-GABA-ONSu: N ^{γ} -Octadecanoyl- γ -aminobutyric acid 2,5-dioxopyrrolidin-1-yl ester
 Pal-Isonip-ONSu: N-Hexadecanoyl-piperidine-4-carboxylic acid 2,5-dioxopyrrolidin-1-yl ester
 Pal-Glu(OBu^t)-ONSu: N ^{α} -Hexadecanoyl-L-glutamic acid α -2,5-dioxopyrrolidin-1-yl ester
 10 γ -t-butyl ester
 HOOC-(CH₂)₆-COONSu: ω -Carboxyheptanoic acid 2,5-dioxopyrrolidin-1-yl ester.
 HOOC-(CH₂)₁₀-COONSu: ω -Carboxyundecanoic acid 2,5-dioxopyrrolidin-1-yl ester.
 HOOC-(CH₂)₁₂-COONSu: ω -Carboxytridecanoic acid 2,5-dioxopyrrolidin-1-yl ester.
 HOOC-(CH₂)₁₄-COONSu: ω -Carboxypentadecanoic acid 2,5-dioxopyrrolidin-1-yl ester.
 15 HOOC-(CH₂)₁₆-COONSu: ω -Carboxyheptadecanoic acid 2,5-dioxopyrrolidin-1-yl ester.
 HOOC-(CH₂)₁₈-COONSu: ω -Carboxynonadecanoic acid 2,5-dioxopyrrolidin-1-yl ester.

Analytical

20 Plasma Desorption Mass Spectrometry

Sample preparation:

- The sample is dissolved in 0.1 % TFA/EtOH (1:1) at a concentration of 1 μ g/ μ l. The sample solution (5-10 μ l) is placed on a nitrocellulose target (Bio-ion AB, Uppsala, Sweden) and allowed to adsorb to the target surface for 2 minutes. The target is subsequently rinsed with 2x25 μ l 0.1 % TFA and spin-dried. Finally, the nitrocellulose target is placed in a target carousel and introduced into the mass spectrometer.

30 MS analysis:

- PDMS analysis was carried out using a Bio-ion 20 time-of flight instrument (Bio-ion Nordic AB, Uppsala, Sweden). An acceleration voltage of 15 kV was applied and molecular ions formed by bombardment of the nitrocellulose surface with 252-Cf fission fragments were accelerated towards a stop detector. The resulting time-of-flight spectrum was calibrated into a true mass spectrum using the H⁺ and NO⁺ ions at m/z 1 and 30, respectively. Mass spectra

were generally accumulated for 1.0×10^6 fission events corresponding to 15-20 minutes. Resulting assigned masses all correspond to isotopically averaged molecular masses. The accuracy of mass assignment is generally better than 0.1 %.

5

MALDI-MS

MALDI-TOF MS analysis was carried out using a Voyager RP instrument (PerSeptive Biosystems Inc., Framingham, MA) equipped with delayed extraction and operated in linear mode. Alpha-cyano-4-hydroxy-cinnamic acid was used as matrix, and mass assignments were based on external calibration.

10

Example 1

Synthesis of N^α-hexadecanoyl-Glu(ONSu)-OBu^t.

15

To a suspension of H-Glu(OH)-OBu^t (4.2 g, 20.6 mmol), DMF (500 ml) and EDPA (2.65 g, 20.6 mmol) was added drop by drop a solution of Pal-ONSu (7.3 g, 20.6 mmol) in DMF (100 ml). The reaction mixture was stirred for 64 h at room temperature and then concentrated *in vacuo* to a total volume of 20 ml. The residue was partitioned between 10% aqueous citric acid (300 ml) and ethyl acetate (250 ml), and the phases were separated. The organic phase was concentrated *in vacuo* and the residue dissolved in DMF (50 ml). The resulting solution was added drop by drop to a 10% aqueous solution of citric acid (500 ml) kept at 0 °C. The precipitated compound was collected and washed with iced water and dried in a vacuum drying oven. The dried compound was dissolved in DMF (45 ml) and HONSu (2.15 g, 18.7 mmol) was added. To the resulting mixture was added a solution of N,N'-dicyclohexylcarbodiimide (3.5 g, 17 mmol) in dichloromethane (67 ml). The reaction mixture was stirred for 16 h at room temperature, and the precipitated compound was filtered off. The precipitate was recrystallised from n-heptane/2-propanol to give the title compound (6.6 g, 72%).

25
30

Example 2

Synthesis of N^α-octadecanoyl-Glu(ONSu)-OBu^t.

To a suspension of H-Glu(OH)-OBu^t (2.82 g, 13.9 mmol), DMF (370 ml) and EDPA (1.79 g, 13.9 mmol) was added drop by drop a solution of Ste-ONSu (5.3 g, 13.9 mmol) in DMF (60 ml). Dichloromethane (35 ml) was added, and the reaction mixture was stirred for 24 h at room temperature and then concentrated *in vacuo*. The residue was partitioned between
5 10% aqueous citric acid (330 ml) and ethyl acetate (200 ml), and the phases were separated. The organic phase was concentrated *in vacuo* and the residue dissolved in DMF (60 ml). The resulting solution was added drop by drop to a 10% aqueous solution of citric acid (400 ml) kept at 0 °C. The precipitated compound was collected and washed with iced water and dried in a vacuum drying oven. The dried compound was dissolved in DMF (40 ml) and
10 HONSu (1.63 g, 14.2 mmol) was added. To the resulting mixture was added a solution of DCC (2.66 g, 12.9 mmol) in dichloromethane (51 ml). The reaction mixture was stirred for 64 h at room temperature, and the precipitated compound was filtered off. The precipitate was recrystallised from n-heptane/2-propanol to give the title compound (4.96 g, 68 %).

15

Example 3

Synthesis of Arg^{26,34}, Lys³⁶ (N^t-(γ-glutamyl(N^α-hexadecanoyl))) GLP-1 (7-36)-OH.

20

To a mixture of Arg^{26,34}, Lys³⁶ GLP-1 (7-36)-OH (12.2 mg, 3.67 μmol), EDPA (13.3 mg, 103 μmol), NMP (1.71 ml) and water (855 μl) was added a solution of Pal-Glu(ONSu)-OBu^t (5.94 mg, 11 μmol), prepared as described above, in NMP (148 μl). The reaction mixture was gently shaken for 5 min. at room temperature, and then allowed to stand for an additional 90
25 min. at room temperature. The reaction was quenched by the addition of a solution of glycine (6 mg, 81 μmol) in water (0.6 ml). A 0.5 % aqueous solution of ammonium-acetate (38 ml) was added, and the resulting mixture eluted onto a Varian 5g C8 Mega Bond Elut®, the immobilised compound washed with 5% aqueous acetonitril (20 ml), and finally liberated from the cartridge by elution with TFA (25 ml). The eluate was concentrated *in vacuo*, and the re-
30 sidue purified by column chromatography using a cyanopropyl column (Zorbax 300SB-CN) and a standard acetonitril/TFA system. The column was heated to 65°C and the acetonitril gradient was 0-100% in 60 minutes. The title compound (3.1 mg, 23 %) was isolated, and the product was analysed by PDMS. The m/z value for the protonated molecular ion was found to be 3695 ± 3. The resulting molecular weight is thus 3694 ± 3 amu (theoretical value
35 3694 amu).

Example 45 **Synthesis of Arg^{26,34},Lys³⁶ (N^ε-(γ-glutamyl(N^α-octadecanoyl))) GLP-1 (7-36)-OH.**

To a mixture of Arg^{26,34},Lys³⁶ GLP-1 (7-36)-OH (12.2 mg, 3.7 μmol), EDPA (13.3 mg, 103 μmol), NMP (1.71 ml) and water (855 μl) was added a solution of Ste-Glu(ONSu)-OBU¹ (6.25
10 mg, 11 μmol), prepared as above, in NMP (1 ml). The reaction mixture was gently shaken for 5 min. at room temperature, and then allowed to stand for an additional 90 min. at room temperature. The reaction was quenched by the addition of a solution of glycine (6 mg, 81 μmol) in water (0.6 ml). A 0.5 % aqueous solution of ammonium acetate (54 ml) was added, and the resulting mixture eluted onto a Varian 5g C8 Mega Bond Elut[®], the immobilised
15 compound washed with 5% aqueous acetonitril (20 ml), and finally liberated from the cartridge by elution with TFA (25 ml). The eluate was concentrated *in vacuo*, and the residue purified by column chromatography using a cyanopropyl column (Zorbax 300SB-CN) and a standard acetonitril/TFA system. The column was heated to 65°C and the acetonitril gradient was 0-100% in 60 minutes. The title compound (3.7 mg, 27 %) was isolated, and the pro-
20 duct was analysed by PDMS. The m/z value for the protonated molecular ion was found to be 3723 ± 3. The resulting molecular weight is thus 3722 ± 3 amu (theoretical value 3722 amu).

Example 525 **Synthesis of Arg¹⁸, Leu²⁰, Gln³⁴, Lys³³ (N^ε-(γ-aminobutyroyl(N^α-hexadecanoyl))) Exendin-4-(7-45)-NH₂.**

To a mixture of Arg¹⁸, Leu²⁰, Gln³⁴-Exendin-4-NH₂ (9.7 mg, 2.3 μmol), EDPA (8.4 mg, 64.7 μmol), NMP (1.36 ml) and water (0.68 ml) was added a solution of Pal-GABA-ONSu (3 mg, 6.9 μmol) in NMP (76 μl). The reaction mixture was gently shaken for 5 min., and then
30 allowed to stand for an additional 90 min. at room temperature. The reaction was quenched by the addition of a solution of glycine (3.8 mg, 50.8 μmol) in water (38 μl). The resulting mixture was purified by column chromatography using a cyanopropyl column (Zorbax 300SB-CN) and a standard acetonitril/TFA system. The column was heated to 65°C and the acetonitril gradient was 0-100% in 60 minutes. The title compound (4.5 mg, 43 %) was iso-
35 lated, and the product was analysed by PDMS. The m/z value for the protonated molecular ion was found to be 4532.8 ± 3. The resulting molecular weight is thus 4531.8 ± 3 amu (theoretical value 4534 amu).

Example 6

Synthesis of Arg³³, Leu²⁰, Gln³⁴, Lys¹⁸ (N^ε-(γ-aminobutyryl(N^α-hexadecanoyl))) Exendin-4-(7-45)-NH₂.

- 5 To a mixture of Arg³³, Leu²⁰, Gln³⁴-Exendin-4-NH₂ (10 mg, 2.4 μmol), EDPA (8.6 mg, 66.5 μmol), NMP (1.4 ml) and water (0.7 ml) was added a solution of Pal-GABA-ONSu (3.1 mg, 7.1 μmol) in NMP (78 μl). The reaction mixture was gently shaken for 5 min., and then allowed to stand for an additional 145 min. at room temperature. The reaction was quenched by the addition of a solution of glycine (3.9 mg, 52.3 μmol) in water (39 μl). The resulting
- 10 mixture was purified by column chromatography using a cyanopropyl column (Zorbax 300SB-CN) and a standard acetonitril/TFA system. The column was heated to 65°C and the acetonitril gradient was 0-100% in 60 minutes. The title compound (2.9 mg, 21 %) was isolated, and the product was analysed by PDMS. The m/z value for the protonated molecular ion was found to be 4533.8 ± 3. The resulting molecular weight is thus 4532.8 ± 3 amu
- 15 (theoretical value 4534 amu).

BIOLOGICAL FINDINGS**Protraction of GLP-1 derivatives after s.c. administration**

- 20 The protraction of a number GLP-1 derivatives of the invention was determined by monitoring the concentration thereof in plasma after sc administration to healthy pigs, using the method described below. For comparison also the concentration in plasma of GLP-1(7-37) after sc. administration was followed. The protraction of other GLP-1 derivatives of the invention can be determined in the same way.

25

- Pigs (50% Duroc, 25% Yorkshire, 25% Danish Landrace, app 40 kg) were fasted from the beginning of the experiment. To each pig 0.5 nmol of test compound per kg body weight was administered in a 50 μM isotonic solution (5 mM phosphate, pH 7.4, 0.02% Tween®-20 (Merck), 45 mg/ml mannitol (pyrogen free, Novo Nordisk). Blood samples were drawn from a
- 30 catheter in vena jugularis. 5 ml of the blood samples were poured into chilled glasses containing 175 μl of the following solution: 0.18 M EDTA, 1500 KIE/ml aprotinin (Novo Nordisk) and 3% bacitracin (Sigma), pH 7.4. Within 30 min, the samples were centrifuged for 10 min at 5-6000*g. Temperature was kept at 4°C. The supernatant was pipetted into different glasses and kept at minus 20°C until use.

The plasma concentrations of the peptides were determined by RIA using a monoclonal antibody specific for the N-terminal region of GLP-1(7-37). The cross reactivities were less than 1% with GLP-1(1-37) and GLP-1(8-36)amide and < 0.1% with GLP-1(9-37), GLP-1(10-36)amide and GLP-1(11-36)amide. The entire procedure was carried out at 4°C.

The assay was carried out as follows: 100 µl plasma was mixed with 271 µl 96% ethanol, mixed using a vortex mixer and centrifuged at 2600*g for 30 min. The supernatant was decanted into Minisorp tubes and evaporated completely (Savant Speedvac AS290). The evaporation residue was reconstituted in the assay buffer consisting of 80 mM NaH₂PO₄/Na₂HPO₄, 0.1 % HSA (Orpha 20/21, Behring), 10 mM EDTA, 0.6 mM thiomersal (Sigma), pH 7.5. Samples were reconstituted in volumes suitable for their expected concentrations, and were allowed to reconstitute for 30 min. To 300 µl sample, 100 µl antibody solution in dilution buffer containing 40 mM NaH₂PO₄/Na₂HPO₄, 0.1 % HSA, 0.6 mM thiomersal, pH 7.5, was added. A non-specific sample was prepared by mixing 300 µl buffer with 100 µl dilution buffer. Individual standards were prepared from freeze dried stocks, dissolved in 300 µl assay buffer. All samples were pre-incubated in Minisorp tubes with antibody as described above for 72 h. 200 µl tracer in dilution buffer containing 6-7000 CPM was added, samples were mixed and incubated for 48 h. 1.5 ml of a suspension of 200 ml per litre of heparin-stabilised bovine plasma and 18 g per litre of activated carbon (Merck) in 40 mM NaH₂PO₄/Na₂HPO₄, 0.6 mM thiomersal, pH 7.5, was added to each tube. Before use, the suspension was mixed and allowed to stand for 2 h at 4°C. All samples were incubated for 1 h at 4°C and then centrifuged at 3400*g for 25 min. Immediately after the centrifugation, the supernatant was decanted and counted in a γ-counter. The concentration in the samples was calculated from individual standard curves. Plasma concentrations were found, calculated as % of the maximum concentration for the individual compounds (n=2). The GLP-1 derivatives of the invention have a protracted profile of action relative to GLP-1(7-37) and are much more persistent in plasma than GLP-1(7-37). The time at which the peak concentration in plasma is achieved varies within wide limits, depending on the particular GLP-1 derivative selected.

Stimulation of cAMP formation in a cell line expressing the cloned human GLP-1 receptor

In order to demonstrate efficacy of the GLP-1 derivatives, their ability to stimulate formation of cAMP in a cell line expressing the cloned human GLP-1 receptor was tested. An EC_{50} was calculated from the dose-response curve.

- 5 Baby hamster kidney (BHK) cells expressing the human pancreatic GLP-1 receptor were used (Knudsen and Pridal, 1996, Eur. J. Pharm. 318, 429-435). Plasma membranes were prepared (Adelhorst *et al*, 1994, J. Biol. Chem. 269, 6275) by homogenisation in buffer (10 mmol/l Tris-HCl and 30 mmol/l NaCl pH 7.4, containing, in addition, 1 mmol/l dithiothreitol, 5 mg/l leupeptin (Sigma, St. Louis, MO, USA), 5 mg/l pepstatin (Sigma, St. Louis, MO, USA), 100 mg/l bacitracin (Sigma, St. Louis, MO, USA), and 16 mg/l aprotinin (Novo Nordisk A/S, Bagsvaerd, Denmark)). The homogenate was centrifuged on top of a layer of 41 w/v% sucrose. The white band between the two layers was diluted in buffer and centrifuged. Plasma membranes were stored at -80°C until used.

- The assay was carried out in 96-well microtiter plates in a total volume of 140 µl. The buffer used was 50 mmol/l Tris-HCl, pH 7.4 with the addition of 1 mmol/l EGTA, 1.5 mmol/l $MgSO_4$, 1.7 mmol/l ATP, 20 mM GTP, 2 mmol/l 3-isobutyl-1-methylxanthine, 0.01 % Tween-20 and 0.1 % human serum albumin (Reinst, Behringwerke AG, Marburg, Germany). Compounds to be tested for agonist activity were dissolved and diluted in buffer, added to the membrane preparation and the mixture was incubated for 2 h at 37°C. The reaction was stopped by the addition of 25 µl of 0.05 mol/l HCl. Samples were diluted 10 fold before analysis for cAMP by a scintillation proximity assay (RPA 538, Amersham, UK).

CLAIMS

1. A derivative of GLP-1(7-C), wherein C is 35 or 36 which derivative has just one lipophilic
5 substituent which is attached to the C-terminal amino acid residue, provided that said derivative is not selected from:
- Arg^{26,34}Lys³⁶(N^ε-(ω-carboxynonadecanoyl))-GLP-1(7-36)-OH,
Arg^{26,34}Lys³⁶(N^ε-(ω-carboxyheptadecanoyl))-GLP-1(7-36)-OH,
Arg^{26,34}Lys³⁶(N^ε-(ω-carboxyundecanoyl))-GLP-1(7-36)-OH,
10 Arg^{26,34}Lys³⁶(N^ε-(ω-carboxyheptanoyl))-GLP-1(7-36)-OH,
Arg^{26,34}Lys³⁶(N^ε-(ω-carboxyheptanoyl))-GLP-1(7-36)-OH.
2. A GLP-1 derivative according to any one of the preceding claims, wherein the lipophilic substituent comprises from 4 to 40 carbon atoms, more preferred from 8 to 25 carbon atoms.
- 15 3. A GLP-1 derivative according to any one of the preceding claims, wherein a lipophilic substituent is attached to an amino acid residue in such a way that a carboxyl group of the lipophilic substituent forms an amide bond with an amino group of the amino acid residue.
- 20 4. A GLP-1 derivative according to any one of the claims 1-2, wherein a lipophilic substituent is attached to an amino acid residue in such a way that an amino group of the lipophilic substituent forms an amide bond with a carboxyl group of the amino acid residue.
5. A GLP-1 derivative according to any one of the preceding claims, wherein the lipophilic substituent is attached to the parent peptide by means of a spacer.
- 25 6. A GLP-1 derivative according to claim 5, wherein the spacer is an unbranched alkane α,ω-dicarboxylic acid group having from 1 to 7 methylene groups, preferably two methylene groups, which form a bridge between an amino group of the parent peptide and an amino group of the
30 lipophilic substituent.
7. A GLP-1 derivative according to claim 5, wherein the spacer is an amino acid residue except Cys, or a dipeptide such as Gly-Lys.

8. A GLP-1 derivative according to claim 7, wherein a carboxyl group of the parent peptide forms an amide bond with an amino group of Lys or a dipeptide containing a Lys residue, and the other amino group of the Lys spacer or a dipeptide spacer containing a Lys residue forms an amide bond with a carboxyl group of the lipophilic substituent.

5

9. A GLP-1 derivative according to claim 7, wherein an amino group of the parent peptide forms an amide bond with a carboxylic group of the amino acid residue or dipeptide spacer, and an amino group of the amino acid residue or dipeptide spacer forms an amide bond with a carboxyl group of the lipophilic substituent.

10

10. A GLP-1 derivative according to claim 7, wherein a carboxyl group of the parent peptide forms an amide bond with an amino group of the amino acid residue spacer or dipeptide spacer, and a carboxyl group of the amino acid residue spacer or dipeptide spacer forms an amide bond with an amino group of the lipophilic substituent.

15

11. A GLP-1 derivative according to claim 7, wherein a carboxyl group of the parent peptide forms an amide bond with an amino group of a spacer which is Asp or Glu, or a dipeptide spacer containing an Asp or Glu residue, and a carboxyl group of the spacer forms an amide bond with an amino group of the lipophilic substituent.

20

12. A GLP-1 derivative according to any one of the preceding claims, wherein the lipophilic substituent comprises a partially or completely hydrogenated cyclopentanophenathrene skeleton.

25

13. A GLP-1 derivative according to any of the claims 1-11, wherein the lipophilic substituent is an straight-chain or branched alkyl group.

14. A GLP-1 derivative according to any of the claims 1-11 wherein the lipophilic substituent is the acyl group of a straight-chain or branched fatty acid.

30

15. A GLP-1 derivative according to claim 14 wherein the acyl group is selected from the group comprising $\text{CH}_3(\text{CH}_2)_n\text{CO}-$, wherein n is 4 to 38, preferably $\text{CH}_3(\text{CH}_2)_6\text{CO}-$, $\text{CH}_3(\text{CH}_2)_8\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{10}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{14}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{16}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{18}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{20}\text{CO}-$ and $\text{CH}_3(\text{CH}_2)_{22}\text{CO}-$.

16. A GLP-1 derivative according to any one of the claims 1-11 wherein the lipophilic substituent is an acyl group of a straight-chain or branched alkane α,ω -dicarboxylic acid.
- 5 17. A GLP-1 derivative according to claim 16 wherein the acyl group is selected from the group comprising $\text{HOOC}(\text{CH}_2)_m\text{CO-}$, wherein m is from 4 to 38, preferably from 4 to 24, more preferably selected from the group comprising $\text{HOOC}(\text{CH}_2)_{14}\text{CO-}$, $\text{HOOC}(\text{CH}_2)_{16}\text{CO-}$, $\text{HOOC}(\text{CH}_2)_{18}\text{CO-}$, $\text{HOOC}(\text{CH}_2)_{20}\text{CO-}$ and $\text{HOOC}(\text{CH}_2)_{22}\text{CO-}$.
- 10 18. A GLP-1 derivative according to any one of the claims 1-11, wherein the lipophilic substituent is a group of the formula $\text{CH}_3(\text{CH}_2)_p((\text{CH}_2)_q\text{COOH})\text{CHNH-CO}(\text{CH}_2)_2\text{CO-}$, wherein p and q are integers and p+q is an integer of from 8 to 33, preferably from 12 to 28.
19. A GLP-1 derivative according to any one of the claims 1-11, wherein the lipophilic substituent is a group of the formula $\text{CH}_3(\text{CH}_2)_r\text{CO-NHCH}(\text{COOH})(\text{CH}_2)_2\text{CO-}$, wherein r is an integer of from 10 to 24.
- 15 20. A GLP-1 derivative according to any one of the claims 1-11, wherein the lipophilic substituent is a group of the formula $\text{CH}_3(\text{CH}_2)_s\text{CO-NHCH}((\text{CH}_2)_2\text{COOH})\text{CO-}$, wherein s is an integer of from 8 to 24.
- 20 21. A GLP-1 derivative according to any one of the claims 1-11, wherein the lipophilic substituent is a group of the formula $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-CO}(\text{CH}_2)_u\text{CH}_3$, wherein u is an integer of from 8 to 18.
- 25 22. A GLP-1 derivative according to any one of the claims 1-11, wherein the lipophilic substituent is a group of the formula $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-COCH}((\text{CH}_2)_2\text{COOH})\text{NH-CO}(\text{CH}_2)_w\text{CH}_3$, wherein w is an integer of from 10 to 16.
- 30 23. A GLP-1 derivative according to any one of the claims 1-11, wherein the lipophilic substituent is a group of the formula $-\text{NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NH-CO}(\text{CH}_2)_x\text{CH}_3$, wherein x is an integer of from 10 to 16.

24. A GLP-1 derivative according to any one of the claims 1-11, wherein the lipophilic substituent is a group of the formula $\text{-NHCH(COOH)(CH}_2\text{)}_y\text{NH-CO(CH}_2\text{)}_2\text{CH(COOH)NH-CO(CH}_2\text{)}_y\text{CH}_3$, wherein y is zero or an integer of from 1 to 22.
- 5 25. A GLP-1 derivative according to any of claims 1-24, wherein the parent peptide is selected from the group comprising GLP-1(1-45) or an analogue or a fragment thereof.
26. A GLP-1 derivative according to claim 25, wherein the parent peptide is selected from the group comprising GLP-1(7-35); GLP-1(7-36); GLP-1(7-36)amide; GLP-1(7-37); GLP-1(7-38);
10 GLP-1(7-39); GLP-1(7-40) and GLP-1(7-41) and an analogue thereof.
27. A GLP-1 derivative according to claim 25, wherein the parent peptide is selected from the group comprising GLP-1(1-35); GLP-1(1-36); GLP-1(1-36)amide; GLP-1(1-37); GLP-1(1-38); GLP-1(1-39); GLP-1(1-40); GLP-1(1-41) and an analogue thereof.
15
28. A GLP-1 derivative according to any of the preceding claims wherein the designation analogue comprises derivatives wherein a total of up to fifteen, preferably up to ten amino acid residues have been exchanged with any α -amino acid residue.
- 20 29. A GLP-1 derivative according to any of the preceding claims wherein the designation analogue comprises derivatives wherein a total of up to fifteen, preferably up to ten amino acid residues have been exchanged with any α -amino acid residue which can be coded for by the genetic code.
- 25 30. A GLP-1 derivative according to any of the preceding claims wherein the designation analogue comprises derivatives wherein a total of up to six amino acid residues have been exchanged with any α -amino acid residue which can be coded for by the genetic code.
31. A GLP-1 derivative according to any of the preceding claims, wherein the parent peptide is
30 selected from the group comprising Arg²⁶-GLP-1(7-37); Arg³⁴-GLP-1(7-37); Lys³⁶-GLP-1(7-37); Arg^{26,34}Lys³⁶-GLP-1(7-37); Arg^{26,34}Lys³⁸GLP-1(7-38); Arg^{26,34}Lys³⁹-GLP-1(7-39); Arg^{26,34}Lys⁴⁰-GLP-1(7-40); Arg²⁶Lys³⁶-GLP-1(7-37); Arg³⁴Lys³⁶-GLP-1(7-37); Arg²⁶Lys³⁹-GLP-1(7-39); Arg³⁴Lys⁴⁰-GLP-1(7-40); Arg^{26,34}Lys^{38,39}-GLP-1(7-39); Arg^{26,34}Lys^{36,40}-GLP-1(7-40); Gly⁸Arg²⁶-GLP-1(7-37); Gly⁸Arg³⁴-GLP-1(7-37); Gly⁸Lys³⁶-GLP-1(7-37); Gly⁸Arg^{26,34}Lys³⁶-GLP-1(7-37);

Gly⁸Arg^{26,34}Lys³⁹-GLP-1(7-39); Gly⁸Arg^{26,34}Lys⁴⁰-GLP-1(7-40); Gly⁸Arg²⁶Lys³⁶-GLP-1(7-37); Gly⁸Arg³⁴Lys³⁶-GLP-1(7-37); Gly⁸Arg²⁶Lys³⁹-GLP-1(7-39); Gly⁸Arg³⁴Lys⁴⁰-GLP-1(7-40); Gly⁸Arg^{26,34}Lys^{36,39}-GLP-1(7-39) and Gly⁸Arg^{26,34}Lys^{36,40}-GLP-1(7-40).

- 5 32. A GLP-1 derivative according to any of the claims 1-31, wherein the parent peptide is selected from the group comprising Arg^{26,34}Lys³⁸GLP-1(7-38); Arg^{26,34}Lys³⁹GLP-1(7-39); Arg^{26,34}Lys⁴⁰GLP-1(7-40); Arg^{26,34}Lys⁴¹GLP-1(7-41); Arg^{26,34}Lys⁴²GLP-1(7-42); Arg^{26,34}Lys⁴³GLP-1(7-43); Arg^{26,34}Lys⁴⁴GLP-1(7-44); Arg^{26,34}Lys⁴⁵GLP-1(7-45); Arg^{26,34}Lys³⁸GLP-1(1-38); Arg^{26,34}Lys³⁹GLP-1(1-39); Arg^{26,34}Lys⁴⁰GLP-1(1-40); Arg^{26,34}Lys⁴¹GLP-1(1-41); Arg^{26,34}Lys⁴²GLP-1(1-42); Arg^{26,34}Lys⁴³GLP-1(1-43); Arg^{26,34}Lys⁴⁴GLP-1(1-44); Arg^{26,34}Lys⁴⁵GLP-1(1-45); Arg^{26,34}Lys³⁸GLP-1(2-38); Arg^{26,34}Lys³⁹GLP-1(2-39); Arg^{26,34}Lys⁴⁰GLP-1(2-40); Arg^{26,34}Lys⁴¹GLP-1(2-41); Arg^{26,34}Lys⁴²GLP-1(2-42); Arg^{26,34}Lys⁴³GLP-1(2-43); Arg^{26,34}Lys⁴⁴GLP-1(2-44); Arg^{26,34}Lys⁴⁵GLP-1(2-45); Arg^{26,34}Lys³⁸GLP-1(3-38); Arg^{26,34}Lys³⁹GLP-1(3-39); Arg^{26,34}Lys⁴⁰GLP-1(3-40); Arg^{26,34}Lys⁴¹GLP-1(3-41); Arg^{26,34}Lys⁴²GLP-1(3-42); Arg^{26,34}Lys⁴³GLP-1(3-43); Arg^{26,34}Lys⁴⁴GLP-1(3-44); Arg^{26,34}Lys⁴⁵GLP-1(3-45); Arg^{26,34}Lys³⁸GLP-1(4-38); Arg^{26,34}Lys³⁹GLP-1(4-39); Arg^{26,34}Lys⁴⁰GLP-1(4-40); Arg^{26,34}Lys⁴¹GLP-1(4-41); Arg^{26,34}Lys⁴²GLP-1(4-42); Arg^{26,34}Lys⁴³GLP-1(4-43); Arg^{26,34}Lys⁴⁴GLP-1(4-44); Arg^{26,34}Lys⁴⁵GLP-1(4-45); Arg^{26,34}Lys³⁸GLP-1(5-38); Arg^{26,34}Lys³⁹GLP-1(5-39); Arg^{26,34}Lys⁴⁰GLP-1(5-40); Arg^{26,34}Lys⁴¹GLP-1(5-41); Arg^{26,34}Lys⁴²GLP-1(5-42); Arg^{26,34}Lys⁴³GLP-1(5-43); Arg^{26,34}Lys⁴⁴GLP-1(5-44); Arg^{26,34}Lys⁴⁵GLP-1(5-45); Arg^{26,34}Lys³⁸GLP-1(6-38); Arg^{26,34}Lys³⁹GLP-1(6-39); Arg^{26,34}Lys⁴⁰GLP-1(6-40); Arg^{26,34}Lys⁴¹GLP-1(6-41); Arg^{26,34}Lys⁴²GLP-1(6-42); Arg^{26,34}Lys⁴³GLP-1(6-43); Arg^{26,34}Lys⁴⁴GLP-1(6-44); Arg^{26,34}Lys⁴⁵GLP-1(6-45); Arg²⁶Lys³⁸GLP-1(1-38); Arg³⁴Lys³⁸GLP-1(1-38); Arg^{26,34}Lys^{36,38}GLP-1(1-38); Arg²⁶Lys³⁸GLP-1(7-38); Arg³⁴Lys³⁸GLP-1(7-38); Arg^{26,34}Lys^{36,38}GLP-1(7-38); Arg^{26,34}Lys³⁸GLP-1(7-38); Arg²⁶Lys³⁹GLP-1(1-39); Arg³⁴Lys³⁹GLP-1(1-39); Arg^{26,34}Lys^{36,39}GLP-1(1-39); Arg²⁶Lys³⁹GLP-1(7-39); Arg³⁴Lys³⁹GLP-1(7-39) and Arg^{26,34}Lys^{36,39}GLP-1(7-39).

33. A pharmaceutical composition comprising a GLP-1 derivative according to the present invention and a pharmaceutically acceptable vehicle or carrier.

34. Use of a GLP-1 derivative according to the present invention for the preparation of a medicament which has a protracted profile of action relative to GLP-1(7-37).

35. Use of a GLP-1 derivative according to the present invention for the preparation of a medicament with a protracted profile of action for the treatment of non-insulin dependent diabetes mellitus.
- 5 36. Use of a GLP-1 derivative according to the present invention for the preparation of a medicament with a protracted profile of action for the treatment of insulin dependent diabetes mellitus.
- 10 37. Use of a GLP-1 derivative according to the present invention for the preparation of a medicament with a protracted profile of action for the treatment of obesity.
38. Use of a GLP-1 derivative according to the present invention for the preparation of a medicament for use in the treatment of diabetes in a regimen which additionally comprises treatment with another antidiabetic agent.
- 15 39. The use according to claim 38, wherein the antidiabetic agent is human insulin or an analogue or a derivative thereof.
40. The use according to claim 38, wherein the antidiabetic agent is an oral hypoglycaemic agent.
- 20 41. The use according to claim 40, wherein the oral hypoglycaemic agent is a sulfonylurea, preferably tolbutamide, glibenclamide, glipizide or gliclazide.
42. The use according to claim 40, wherein the oral hypoglycaemic agent is a biguanide, preferably metformin.
43. The use according to claim 40, wherein the oral hypoglycaemic agent is a thiazolidinedione, preferably troglitazone or ciglitazone.
- 25 44. The use according to claim 40, wherein the oral hypoglycaemic agent is a glucosidase inhibitor, preferably acarbose.
45. The use according to claim 40, wherein the oral hypoglycaemic agent is an agent acting on the ATP-dependent potassium channel of the β -cells, preferably glibenclamide, glipizide, gliclazide or repaglinide.

46. The use according to any one of claims 38 to 45, wherein the GLP-1 derivative and said other antidiabetic agent are administered so as to obtain a synergistic effect.
47. An exendin derivative wherein at least one amino acid residue of the parent peptide has a lipophilic substituent attached.
- 5
48. An exendin derivative according to claim 47, wherein only one lipophilic substituent is present.
49. An exendin derivative according to claim 48, wherein the lipophilic substituent is attached to the N-terminal amino acid residue.
- 10
50. An exendin derivative according to claim 48, wherein the lipophilic substituent is attached to the C-terminal amino acid residue.
51. An exendin derivative according to claim 48, wherein the lipophilic substituent is attached to an amino acid residue which is not the N-terminal or C-terminal amino acid residue.
- 15
52. An exendin derivative according to claim 47, wherein two lipophilic substituents are present.
- 20
53. An exendin derivative according to claim 52, wherein one of the lipophilic substituents is attached to the N-terminal amino acid residue while the other is attached to the C-terminal amino acid residue.
54. An exendin derivative according to claim 52, wherein one of the lipophilic substituents is attached to the C-terminal amino acid residue while the other is attached to an amino acid residue which is not the N-terminal or C-terminal amino acid residue.
- 25
55. An exendin derivative according to claim 52, wherein both lipophilic substituents are attached to amino acid residues which are neither the N-terminal nor the C-terminal amino acid residue.
- 30
56. An exendin derivative according to any one of claims 47 to 55, wherein the lipophilic substituent comprises from 4 to 40 carbon atoms, more preferred from 8 to 25 carbon atoms.

57. An exendin derivative according to any one of claims 47 to 56, wherein a lipophilic substituent is attached to an amino acid residue in such a way that a carboxyl group of the lipophilic substituent forms an amide bond with an amino group of the amino acid residue.

5

58. An exendin derivative according to any one of the claims 47 to 56, wherein a lipophilic substituent is attached to an amino acid residue in such a way that an amino group of the lipophilic substituent forms an amide bond with a carboxyl group of the amino acid residue.

10 59. An exendin derivative according to any one of the claims 47 to 58, wherein the lipophilic substituent is attached to the parent peptide by means of a spacer.

60. An exendin derivative according to claim 59, wherein the spacer is an unbranched alkane α,ω -dicarboxylic acid group having from 1 to 7 methylene groups, preferably two methylene groups, which form a bridge between an amino group of the parent peptide and an amino group of the lipophilic substituent.

15

61. An exendin derivative according to claim 59, wherein the spacer is an amino acid residue except cys, or a dipeptide such as gly-lys.

20

62. An exendin derivative according to claim 59, wherein a carboxyl group of the parent peptide forms an amide bond with an amino group of lys or a dipeptide containing a lys residue, and the other amino group of the lys spacer or a dipeptide spacer containing a lys residue forms an amide bond with a carboxyl group of the lipophilic substituent.

25

63. An exendin derivative according to claim 59, wherein an amino group of the parent peptide forms an amide bond with a carboxylic group of the amino acid residue or dipeptide spacer, and an amino group of the amino acid residue or dipeptide spacer forms an amide bond with a carboxyl group of the lipophilic substituent.

30

64. An exendin derivative according to claim 59, wherein a carboxyl group of the parent peptide forms an amide bond with an amino group of the amino acid residue spacer or dipeptide spacer, and a carboxyl group of the amino acid residue spacer or dipeptide spacer forms an amide bond with an amino group of the lipophilic substituent.

65. An extendin derivative according to claim 59, wherein a carboxyl group of the parent peptide forms an amide bond with an amino group of a spacer which is asp or glu, or a dipeptide spacer containing an asp or glu residue, and a carboxyl group of the spacer forms an amide bond with an amino group of the lipophilic substituent.
66. An extendin derivative according to any one the claims 47 to 65, wherein the lipophilic substituent comprises a partially or completely hydrogenated cyclopentanophenathrene skeleton.
67. An extendin derivative according to any of the claims 47 to 65, wherein the lipophilic substituent is an straight-chain or branched alkyl group.
68. An extendin derivative according to any of the claims 47 to 65, wherein the lipophilic substituent is the acyl group of a straight-chain or branched fatty acid.
69. An extendin derivative according to claim 68 wherein the acyl group is selected from the group comprising $\text{CH}_3(\text{CH}_2)_n\text{CO}-$, wherein n is 4 to 38, preferably $\text{CH}_3(\text{CH}_2)_6\text{CO}-$, $\text{CH}_3(\text{CH}_2)_8\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{10}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{12}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{14}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{16}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{18}\text{CO}-$, $\text{CH}_3(\text{CH}_2)_{20}\text{CO}-$ and $\text{CH}_3(\text{CH}_2)_{22}\text{CO}-$.
70. An extendin derivative according to any one of the claims 47 to 65 wherein the lipophilic substituent is an acyl group of a straight-chain or branched alkane α,ω -dicarboxylic acid.
71. An extendin derivative according to claim 70 wherein the acyl group is selected from the group comprising $\text{HOOC}(\text{CH}_2)_m\text{CO}-$, wherein m is from 4 to 38, preferably from 4 to 24, more preferred selected from the group comprising $\text{HOOC}(\text{CH}_2)_{14}\text{CO}-$, $\text{HOOC}(\text{CH}_2)_{16}\text{CO}-$, $\text{HOOC}(\text{CH}_2)_{18}\text{CO}-$, $\text{HOOC}(\text{CH}_2)_{20}\text{CO}-$ and $\text{HOOC}(\text{CH}_2)_{22}\text{CO}-$.
72. An extendin derivative according to any one of the claims 47 to 65, wherein the lipophilic substituent is a group of the formula $\text{CH}_3(\text{CH}_2)_p((\text{CH}_2)_q\text{COOH})\text{CHNH-CO}(\text{CH}_2)_2\text{CO}-$, wherein p and q are integers and p+q is an integer of from 8 to 33, preferably from 12 to 28.

73. An exendin derivative according to any one of the claims 47 to 65, wherein the lipophilic substituent is a group of the formula $\text{CH}_3(\text{CH}_2)_r\text{CO-NHCH}(\text{COOH})(\text{CH}_2)_2\text{CO-}$, wherein r is an integer of from 10 to 24.

5 74. An exendin derivative according to any one of the claims 47 to 65, wherein the lipophilic substituent is a group of the formula $\text{CH}_3(\text{CH}_2)_s\text{CO-NHCH}((\text{CH}_2)_2\text{COOH})\text{CO-}$, wherein s is an integer of from 8 to 24.

75. An exendin derivative according to any one of the claims 47 to 65, wherein the lipophilic
10 substituent is a group of the formula $\text{-NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-CO}(\text{CH}_2)_u\text{CH}_3$, wherein u is an integer of from 8 to 18.

76. An exendin derivative according to any one of the claims 47 to 65, wherein the lipophilic substituent is a group of the formula $\text{-NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-COCH}((\text{CH}_2)_2\text{COOH})\text{NH-}$
15 $\text{CO}(\text{CH}_2)_w\text{CH}_3$, wherein w is an integer of from 10 to 16.

77. An exendin derivative according to any one of the claims 47 to 65, wherein the lipophilic substituent is a group of the formula $\text{-NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NH-}$
20 $\text{CO}(\text{CH}_2)_x\text{CH}_3$, wherein x is an integer of from 10 to 16.

78. An exendin derivative according to any one of the claims 47 to 65, wherein the lipophilic substituent is a group of the formula $\text{-NHCH}(\text{COOH})(\text{CH}_2)_4\text{NH-CO}(\text{CH}_2)_2\text{CH}(\text{COOH})\text{NH-}$
 $\text{CO}(\text{CH}_2)_y\text{CH}_3$, wherein y is zero or an integer of from 1 to 22.

25 79. An exendin derivative according to any of the claims 47 to 78, wherein the designation analogue comprises derivatives wherein a total of up to fifteen, preferably up to ten amino acid residues have been exchanged with any α -amino acid residue.

80. An exendin derivative according to any of the claims 47 to 79, wherein the designation
30 analogue comprises derivatives wherein a total of up to fifteen, preferably up to ten amino acid residues have been exchanged with any α -amino acid residue which can be coded for by the genetic code.

81. An exendin derivative according to any of the claims 47 to 80, wherein the designation analogue comprises derivatives wherein a total of up to six amino acid residues have been exchanged with any α -amino acid residue which can be coded for by the genetic code.
- 5 82. An exendin derivative according to any of the claims 47 to 81, wherein the parent peptide is HGEGTFTSDLSKQMEEEEAVRLFIEWLKNGGX, wherein X = P or Y, or a fragment or an analogue thereof.
83. An exendin derivative according to any of claims 47 to 81, wherein the parent peptide is
10 HX1X2GTFITSDLSKQMEEEEAVRLFIEWLKNGGPSSGAPPPS, wherein X1X2 = SD or GE, or a fragment or an analogue thereof.
84. An exendin derivative according to any of claims 47 to 81, wherein the parent peptide is
15 DLSKQMEEEEAVRLFIEWLKNGGPSSGAPPPS, or a fragment or an analogue thereof.
85. An exendin derivative according to claim 47, which is selected from
Arg¹⁸, Leu²⁰, Gln³⁴, Lys³³ (N^ε-(γ -aminobutyryl(N^α-hexadecanoyl))) Exendin-4-(7-45)-NH₂,
Arg³³, Leu²⁰, Gln³⁴, Lys¹⁸ (N^ε-(γ -aminobutyryl(N^α-hexadecanoyl))) Exendin-4-(7-45)-NH₂.
- 20 86. A pharmaceutical composition comprising an exendin derivative according to the present invention and a pharmaceutically acceptable vehicle or carrier.
87. Use of an exendin derivative according to the present invention for the preparation of a medicament which has a protracted profile of action relative to exendin.
- 25 88. Use of an exendin derivative according to the present invention for the preparation of a medicament with a protracted profile of action for the treatment of non-insulin dependent diabetes mellitus.
- 30 89. Use of an exendin derivative according to the present invention for the preparation of a medicament with a protracted profile of action for the treatment of insulin dependent diabetes mellitus.

90. Use of an exendin derivative according to the present invention for the preparation of a medicament with a protracted profile of action for the treatment of obesity.

91. A method of treating insulin dependent or non-insulin dependent diabetes mellitus in a patient in need of such a treatment, comprising administering to the patient a therapeutically effective amount of a exendin derivative according to the present invention together with a pharmaceutically acceptable carrier.

Novo Nordisk A/S

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 99/00086

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/605, A61K 38/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, MEDLINE, EMBASE, CA, SCISEARCH, DERWENT DRUG FILE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 9808871 A1 (NOVO NORDISK A/S), 5 March 1998 (05.03.98) --	1-46
X	WO 9629342 A1 (NOVO NORDISK A/S), 26 Sept 1996 (26.09.96), page 2, line 1 - page 4, line 32 --	1-91
X	US 5614492 A (JOEL F. HABENER), 25 March 1997 (25.03.97), column 3, line 28 - column 4, line 10; column 6, line 48 - column 7, line 61 --	1-46
A	US 5545618 A (DOUGLAS I. BUCKLEY ET AL), 13 August 1996 (13.08.96), abstract, claims --	1-46

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

15 April 1999

Date of mailing of the international search report

05-05-1999

Name and mailing address of the ISA

Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer

Hampus Rystedt
Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00086

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9011296 A1 (THE GENERAL HOSPITAL CORPORATION), 4 October 1990 (04.10.90) --	1-46
A	WO 8706941 A1 (THE GENERAL HOSPITAL CORPORATION), 19 November 1987 (19.11.87) --	1-46
A	US 5631224 A (SUAD EFENDIC ET AL), 20 May 1997 (20.05.97) --	38-46
A	WO 9746584 A1 (BOEHRINGER MANNHEIM GMBH), 11 December 1997 (11.12.97), abstract, claims --	47-91
A	US 5424286 A (JOHN ENG), 13 June 1995 (13.06.95), column 4, line 6 - column 5, line 39 --	47-91
A	WO 9426778 A1 (PROTEIN DELIVERY, INC.), 24 November 1994 (24.11.94), See esp. page 8, line 32 - page 9, line 2, page 11, line 19 - line 26 -- -----	1-91

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 99/00086

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 46, 91
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 46 and 91 relate to methods for treatment of the human body,
a search has been carried out based on the alleged effects of the claimed compounds.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such
an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See next sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 99/00086

The present application relates to two groups of inventions, namely:

1. Derivatives of GLP-1 with a protracted profile of action
2. Derivatives of exendin with a protracted profile of action

The protracted profile of action is achieved by attaching a lipophilic group to one amino acid residue, preferably the C-terminal. As this method is known through WO, 9629342, A1, and as the uses of GLP-1 and of exendin in pharmaceutical compositions are also known in the art, the two groups of inventions are not linked together by a special technical feature as required by PCT Rule 13.2.

Furthermore, the first group of inventions relate to a large number of peptide derivatives technically linked together by their homologies to GLP-1 and the presence of a lipophilic substituent on at least one Lys-residue, giving a protracted profile of action. Derivatives of GLP-1, with the same effects as the claimed derivatives, are well known in the prior art, see e.g. US, 5614492, A. The method of introducing lipophilic substituents in order to obtain a protracted profile of action is also known, see WO, 9629342, A1.

No new effect of the claimed GLP-1 derivatives has been shown to arise from a common technical feature of the derivatives, structural or other, which defines a contribution over the prior art. Each new GLP-1 derivative is therefore considered to be a unique invention according to PCT Rule 13.1 and 13.2.

As all inventions could be searched within one fee, the exact number of inventions has not been calculated.

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/03/99

International application No.

PCT/DK 99/00086

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9808871 A1	05/03/98	AU 3847897 A AU 4112497 A WO 9808872 A	19/03/98 19/03/98 05/03/98
WO 9629342 A1	26/09/96	AU 4939596 A BR 9607669 A CA 2215739 A CN 1181760 A CZ 9702877 A EP 0815135 A NO 974269 A PL 322254 A US 5869602 A	08/10/96 16/06/98 26/09/96 13/05/98 15/04/98 07/01/98 14/11/97 19/01/98 09/02/99
US 5614492 A	25/03/97	US 5118666 A AT 110083 T DE 3750402 D,T EP 0305387 A,B SE 0305387 T3 EP 0587255 A JP 1502746 T JP 2583257 B US 5120712 A WO 8706941 A	02/06/92 15/09/94 01/12/94 08/03/89 16/03/94 21/09/89 19/02/97 09/06/92 19/11/87
US 5545618 A	13/08/96	AT 164852 T CA 2073856 A DE 69129226 D,T DK 512042 T EP 0512042 A,B SE 0512042 T3 ES 2113879 T WO 9111457 A	15/04/98 25/07/91 30/07/98 11/05/98 11/11/92 16/05/98 08/08/91
WO 9011296 A1	04/10/90	EP 0464022 A JP 4504246 T	08/01/92 30/07/92

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/03/99

International application No.

PCT/DK 99/00086

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8706941 A1	19/11/87	AT 110083 T	15/09/94
		DE 3750402 D,T	01/12/94
		EP 0305387 A,B	08/03/89
		SE 0305387 T3	
		EP 0587255 A	16/03/94
		JP 1502746 T	21/09/89
		JP 2583257 B	19/02/97
		US 5118666 A	02/06/92
		US 5120712 A	09/06/92
		US 5614492 A	25/03/97
US 5631224 A	20/05/97	AU 3888893 A	21/10/93
		CN 1088835 A	06/07/94
		EP 0631505 A	04/01/95
		JP 7504670 T	25/05/95
		WO 9318786 A	30/09/93
		DK 9300099 U	13/04/93
WO 9746584 A1	11/12/97	AU 3173297 A	05/01/98
		DE 19622502 A	02/01/98
		DE 19637230 A	19/03/98
US 5424286 A	13/06/95	NONE	
WO 9426778 A1	24/11/94	AU 694919 B	06/08/98
		AU 6946694 A	12/12/94
		CA 2162366 A	24/11/94
		EP 0707596 A	24/04/96
		IL 109619 D	00/00/00
		JP 8510255 T	29/10/96
		US 5359030 A	25/10/94
		US 5438040 A	01/08/95
		US 5681811 A	28/10/97